"EVALUATION OF ENZYME MARKERS IN CARCINOMA CERVIX - A CROSS SECTIONAL STUDY"

By

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Date:

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LIST OF ABBREVIATIONS USED

ΔA/Min	-	Mean absorbance change per minute
Abs	-	Absorbance
ADA	-	Adenosine deaminase
ALP	-	Alkaline phosphatase
ASC-US	-	Atypical squamous cells of undetermined significance.
ASR	-	Age standardized
ATP	-	Adenosine triphosphate
CEA	-	Carcinoembryonic antigen
CIN	-	Cervical intraepithelial neoplasia
CT	-	Computed tomography
Cx	-	Cervix
DM	-	Diabetes mellitus
DNA	-	Deoxyribo nucleic acid
DOA	-	Date of admission
D/W	-	Distilled water
EHNA	-	Erythro-9-(2-hydroxy-3-nonyl) adenine
E proteins	-	Early proteins
Gm	-	Gram
GGT	-	Gamma glutamyl tranferase
HIV	-	Human immuno deficiency virus
Hist	-	Histopathology
HPV	-	Human papilloma virus
HSIL	-	High grade squamous intraepithelial lesion

HSV	-	Herpes simplex virus
HTN	-	Hypertension
H/o	-	History of
IP / OP No.	-	In patient / Out patient Number
IU	-	International units
IVP	-	Intravenous pyelogram
LMP	-	Last menstrual period
L proteins	-	Late proteins
LSIL	-	Low grade squamous intraepithelial lesion
MI	-	Myocardial infarction
min	-	Minute
MDA	-	Malondialdehyde
ml	-	Milli Litre
MRI	-	Magnetic resonance imaging
NADP +	-	Nicotinamide adenine dinucleotide phosphate
NH_3	-	Ammonia
P/A	-	Per abdominal
P/S	-	Per speculum
P/V	-	Per vaginal
Pa MC	-	Past menstrual cycle
PALP	-	Placental alkaline phosphatase
PAM	-	Pregnancy associated macroglobulin
Pr MC	-	Present menstrual cycle
Rb	-	Retinoblastoma
RR	-	Respiratory rate

S	-	Standard
SB	-	Sample blank
SCC	-	Squamous cell carcinoma
SCID	-	Severe combined immunodeficiency syndrome
SCJ	-	Squamous columnar junction
S.D.	-	Standard deviation
Sl. No.	-	Serial Number
S Phase	-	Synthesis phase
T	-	Test
TA	-	Tumor antigen
TB	-	Tuberculosis
Temp	-	Temperature
U/L	-	Unit / Litre
WDPV	-	White discharge per vagina
Yrs	-	Years

ABSTRACT

Background and Objectives

Cervical cancer is the commonest cancer among the Indian women, accounting for a quarter of the global burden of cervical cancer. The disease process is certainly curable if it is diagnosed early. The objective of the study was to assess the reliability of enzyme markers, serum adenosine deaminase (ADA) and gamma glutamyl transferase (GGT) in cervical cancer patients as supportive parameters for diagnostic purpose.

Methods

The present cross-sectional study comprised of 40 healthy female in the age group of 35 to 65 years as controls and 40 cases of clinically and histopathologically confirmed patients of cervical cancer of the same age group, admitted at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre and Cancer Hospital, Belgaum over a period of one year during May-2007 to May-2008. Serum enzyme markers, ADA and GGT were evaluated.

Results

In the present study, mean serum adenosine deaminase level in controls was 20.81 ± 5.97 U/L while in the patients of cervical cancer it was 56.45 ± 19 U/L. The mean serum gamma glutamyl transferase level was 19.61 ± 5.80 U/L in controls whereas in cervical cancer patients it was 40.96 ± 8.45 U/L.

Interpretation and conclusion

The present study revealed that, a significant increase in serum ADA was observed in cervical cancer as compared to controls, $p < 0.001$. An interstage comparison showed a non significant increase in serum ADA levels from stage I to III and a significant increase in serum GGT was observed in cervical cancer as compared to controls, $p < 0.001$. An interstage comparison showed a non significant increase in serum GGT levels from stage I to III.

The study concludes that, serum ADA and GGT may be used as supportive biochemical parameters for diagnostic purpose and may add further for prognostic information in cervical cancer.

Keywords

Cervical cancer; Adenosine deaminase; Gamma glutamyl transferase.

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INTRODUCTION

When we think of cancer in general terms, we are apt to conjure up a process characterized by a steady, remorseless and inexorable progress in which the disease is all conquering and none of the immunological and other defensive forces will help leading to faltering footsteps to the grave.

Cervical cancer is the second most common cancer in women worldwide and represents 12% of all female malignancies. It is estimated that there are 500,000 new cases diagnosed and 250,000 death each year of which 79% occur in developing countries.[1,2] Cervical cancer is the commonest cancer among the Indian women 126,000 new cases 71,000 death around the year 2000, accounting for a quarter of the global burden of cervical cancer.[3,4] The incidence data indicate a rate of approximately eight to 10 per 100,000 per year.[5] Cervical cancer tends to occur during midlife in women, with half of the patients diagnosed between 25 to 65 years of age, rarely affects women under the age of 20.[6]

Risk factor for cervical carcinoma include human papilloma virus (HPV). The role of HPV infection in the oncogenesis of cervical carcinoma is explained to a large degree by the regulation and function of two oncogenes early proteins (E_6 and E_7 proteins). HPV infection (HPV – 16, 18, 31, 45 and 56) has been reported to be associated with 77% of high cervical intraepithelial neoplasia (CIN) and 84% to 100% of invasive cancer.[7]

Studies have suggested that cancer of the cervix is more frequent in;

- Early age at first intercourse.
- Multiple partners.
- Sexually transmitted diseases, HPV, human immunodeficiency virus (HIV), herpes simplex virus (HSV).
- Oral contraceptive use.
- Cigarette smoking.
- Low socioeconomic status.[8,5]

Under nutrition is one of the cause of carcinoma cervix in developing areas of the world. Orr reported abnormal vitamin levels were commonly present in patients with cervical cancer when compared with control values, levels of plasma folate, beta carotene and vitamin C were significantly lower in patients with cervical cancer.[5]

As there is no population based screening programme in India, 70 to 80% of the cervical cancer patients are diagnosed at advanced stages (stage III and IV), with very poor long term survival.[9]

Surgery, radiation or chemoradiation therapies are the only available treatment for cancer cervix, are not accessible to all the victims in the developing countries and when available, women suffer from severe morbidities with overall survival rate of 40%. Hence prevention is the most primary approach to control the disease. The lower incidence in developed country is mainly attributed to the cancer screening programme and treatment of precancerous lesions.[8]

An increase in the activities of enzymes in the serum is indicative of cell damage or overproduction in a tissue rich in the enzymes concerned.[10] In necrotic

conditions, cell destruction leads to the appearance of both mitochondrial and cell sap enzymes in the serum.

Enzymes are present in much higher concentration inside cells and are released into the systemic circulation as a result of tumor necrosis or a change in the membrane permeability of the cancer cells. With few exceptions, most enzymes are not unique for a specific organ; therefore enzymes are most suitable as non specific tumor markers. Elevated enzyme levels may signal the presence of malignancy.[11]

Potential uses of tumor markers are cancer screening in general populations, staging of cancer, prognostic indicator of disease progression and in monitoring of responses to therapy. The most useful serum marker in patients with squamous cell carcinoma (SCC) of the cervix is the SCC antigen initially isolated by Kato and Torigoe in 1977. Although this antigen is not tumor specific, it has been useful as a means to monitor treatment response and to predict tumor recurrence.[12] Similar results have been reported for carcinoembryogenic antigen (CEA) in patients with endocervical adenocarcinoma[13] and placental alkaline phosphatase (PALP).[14] However the analytical method for determination of these need well established centres and are expensive.

As there are very few studies on enzyme markers in cervical cancer the present study has been undertaken to assess the reliability of some of the enzyme markers namely, serum adenosine deaminase (ADA) and gamma glutamyl transferase (GGT) which are inexpensive, analysed by easy methods and may be used as supportive parameters for diagnostic purpose and may add further for prognostic information.

OBJECTIVES

The objectives of the present study were;

1. To estimate serum adenosine deaminase (ADA) and gamma glutamyl transferase (GGT) activity in carcinoma cervix patients.

2. To assess the reliability of serum adenosine deaminase (ADA) and gamma glutamyl transferase (GGT) in carcinoma cervix patients as supportive parameters for diagnostic purpose.

REVIEW OF LITERATURE

The word “cancer” is derived from the Latin for Crab because a cancer adheres to any part of that it seizes up in an obstinate manner like the crab.

As defined by eminent British Oncologist Willis “Cancer is an abnormal mass of tissue the growth of which exceeds and is in-coordinated with that of the normal tissue and persists in the same excessive manner after cessation of the stimuli which evoked the change”.[15]

“Cancer of the uterine cervix is now regarded as a preventable disease”. This statement by a World Health Organization Cancer Committee in 1963 was a milestone in the history of uterine cancer.[16]

Cancer of the uterine cervix is largely a preventable disease that is characterized by a long lead time; precancerous lesions gradually progress through recognizable stages before developing into invasive disease.[17,18,19,20,21,22,23]

The disease process is almost certainly curable if it is identified before its progression to invasive cancer. However invasive cervical cancer remains a disease of significant morbidity, and it is a major cause of cancer deaths in women worldwide, although the incidence and mortality rates of invasive cervical cancer have declined substantially (particularly in countries that have well-developed screening programs.) Cancer of the cervix in its early stages is readily managed with surgery. Radiation or chemoradiation therapies are reserved for high risk early stages or advanced disease.[24,25]

HISTORICAL BACKGROUND OF CERVICAL CANCER

400 BCE:	Hippocrates: Cervical cancer incurable.[26]
1910	Rubin: described 3 patients with incipient carcinoma of the cervix.
1925	Hans Hinselmann: Invented Colposcope.
1928	George Papanicolaou: developed Pap technique.
1932	Broders: gave descriptive name of carcinoma insitu.
1933, 1936, 1938	Te Lindi: first conceived the idea that intra epithelial changes could be the beginning of invasive cancer.
1941	George Papanicolaou and Trout: Pap screening.
1944, 1949	To Linde and Galvin: recognized basal cell hyperplasia and carcinoma in situ.
1947, 1954	Ayre: Spatula technique to prepare cervical smears.
1951, 1953, 1956	Grunberger and Antonie: Designed Colpomicroscopy.
1957, 1965	Younge: Schiller test
1957	Loo et al and Rall et al: Tetracycline fluorescent test.
1959	Anderson: stated that the Biopsy is necessary for diagnosis and is often the only treatment required.
1964	Bonham: Devised enzyme screening test.[16]
1976	Zur Hausen and Gisami: found HPV DNA in cervical cancer and warts.[27]

1988	Bethesda System for Pap results developed.[16]
1990	Improved sampling methods by liquid based cytology and computer based reading systems.[28]

Readers interested in exploring new techniques of integrating intracellular structure and behaviour by electron microscopy, histochemistry, and histophysics are reffered to the following publications;

Electron microscopy: Coman et al. (1955); Howatson and Ham (1957); Lubel et al (1959); Sirtori and Morano (1963).

Histochemistry: McManus and Findley (1949); Foraker and Denham (1957); Pitot (1961); Willinghaghen (1961).

Histophysics: Mellors et al (1952, 1953).[16]

CERVICAL CANCER

Incidence

Worldwide, invasive cervical cancer is the most common genital female malignancy and the second most common malignancy in women, after breast cancer and represents 12% of all female malignancies.[29] It is estimated there are 500,000 new cases diagnosed and 250,000 deaths each year of which 79% occur in developing countries.[1,2]

Cervical cancer is the commonest cancer among the Indian women with 126,000 new cases 71,000 death around the year 2000, accounting for a quarter of the global burden of cervical cancer.[3,4] Incidence data indicate a rate of approximately eight to 10 per 100,000 per year.[5]

For each death from cancer of the cervix, it has been estimated that between 14 and 20 potential years of life before 70 years of age are lost. Assuming, therefore, an average of about 17 years of life lost per death, this gives an estimate of more than 3.4 million women years of life before 70 years of age lost due to cancer of the cervix each year worldwide.[30]

Geographical distribution of cancer of the cervix

The variation in estimated crude incidence rates of invasive cancer of the cervix in the 24 United Nations areas of the world was described by Parkin et al.[31] The highest rates of cancer of the cervix, with corresponding relative frequencies of between 20% and 30% (compared with an estimated 15% worldwide), were observed to occur in the developing areas of the world,

particularly parts of Asia, South America and Africa. Intermediate rates were particularly evident in areas of eastern, northern and Western Europe, while the lowest rates were seen in Australia and New Zealand, Southern Europe, North America and Western Asia.[30]

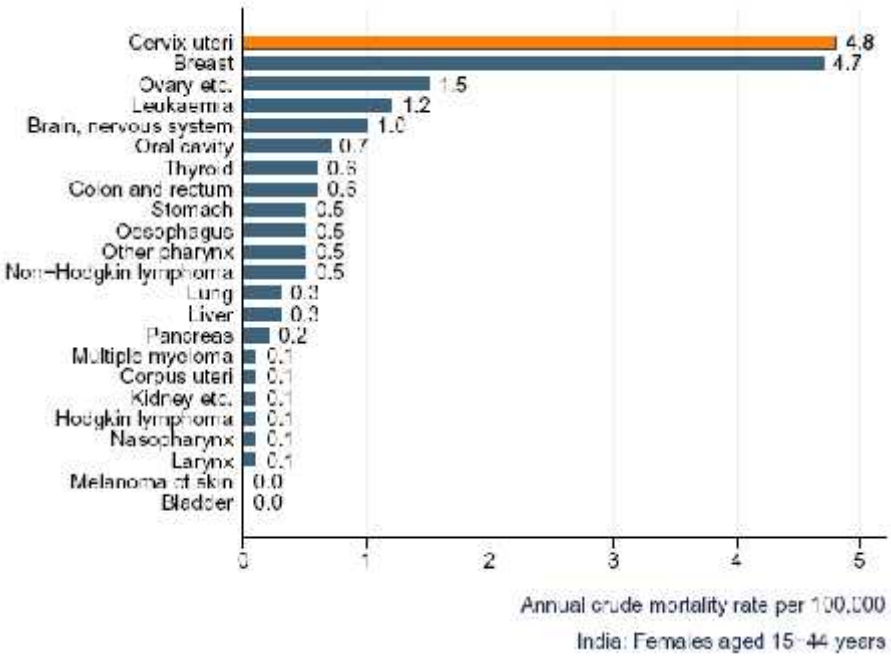


Figure 1: Specific cervical cancer mortality in India compared to other cancers in women of 15 to 44 years of age[32]

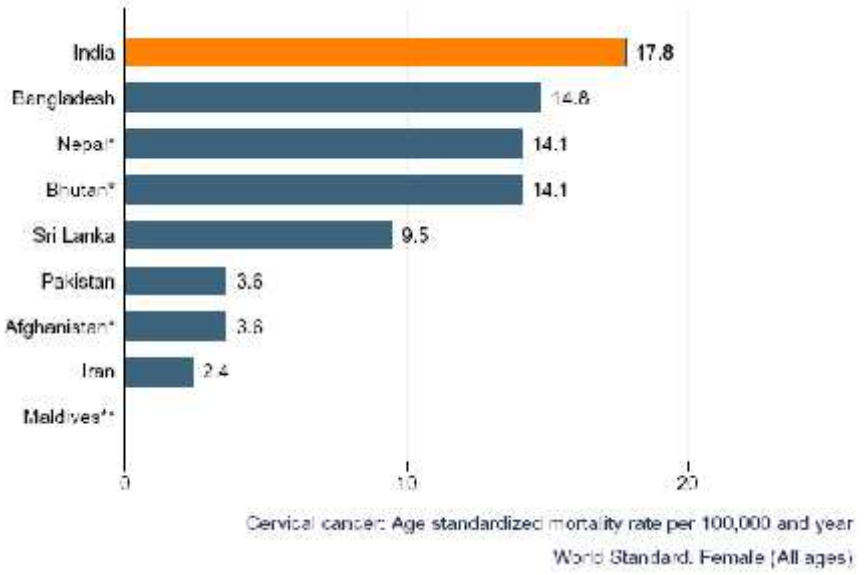


Figure 2: Cervical cancer age standardized (ASR) mortality rates in countries of Southern Asia.[32]

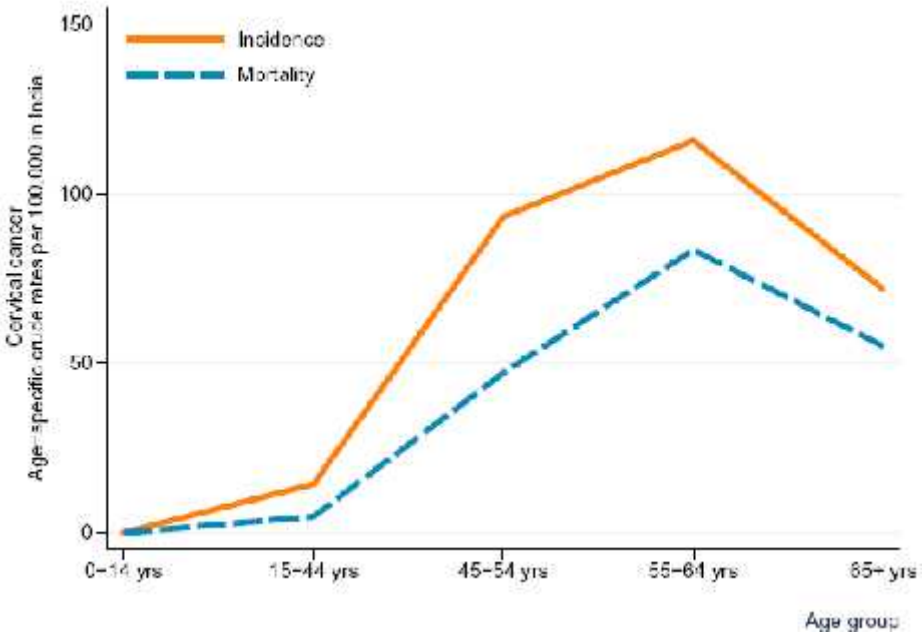


Figure 3: A Comparison of age-specific incidence rates and age-specific mortality rates of cervical cancer in India.[32]

ETIOLOGY

Risk factors for cervical cancer include;

a. Sexual history
b. Smoking
c. Contraception
d. Nutrition
e. Infective agents &
f. Role of Molecular biology in cervical cancer

Sexual history

The oft-quoted statement that nuns and virgins are immune from cervical cancer has been challenged in recent years. More critical examination of the evidence suggests that this contention is unlikely to be true (Griffiths 1991).

Two factors related to sexual behavior – age at first intercourse and number of sexual patterns - have been examined extensively. Rotkin (1973) reported a 50% excess of cases of cervical neoplasia in women who started sexual intercourse before their 20th birthday, supporting the proposal that the adolescent cervix may be more vulnerable to potential oncogenic agents. Several other studies have found a history of multiple sexual partners to be more significant than early age of first intercourse. Harris et al (1980) recorded a 14.2 – fold increased risk of developing severe dysplasia with a past history of six or more partners, and this finding have been confirmed by others. There appears to be little correlation between the number of sexual acts and cervical neoplasia among women with two or less lifetime patterns (Harris et al 1980).[33]

Smoking

A strong epidemiological link between smoking and cervical neoplasia has been shown in studies which have controlled adequately for sexual behavior. A 12.7 fold greater risk of developing carcinoma-in-situ has been found after 12 years of smoking. The products of smoking, cotinine and nicotine, are found in higher concentrations in cervical mucus than in serum from women with CIN. However, little is known about the direct effects of these agents on human epithelia. There are few data on the role of nitrosamines, the chemical carcinogens linked with the development of other smoking- related cancers, in the cervix.

Smoking reduces the number of Langerhans' cells present in the cervical epithelium in a dose dependent manner. These cells, originally derived from

macrophages, play a role in local immune surveillance within epithelia. Because their role is not fully understood, it is difficult to speculate upon the ways in which the reductions of Langerhans' cells may contribute to cervical neoplasia.[33]

Contraception

No definite association between oral contraception and cervical neoplasia has been clearly demonstrated. Studies which have shown an increased risk in oral contraceptive pill users have often failed to control adequately for the sexual history of the women involved (Beral et al, 1988). A lower incidence of cervical neoplasia has been demonstrated in women using a diaphragm, suggesting that a transmissible agent may be involved.[33]

Nutrition

Orr reported abnormal vitamin levels were more commonly present in patients with cervical cancer. When compared with control values, levels of plasma folate, beta carotene and vitamin C were significantly lower in patients with cervical cancer.[5]

Infective Agents

Agents that are known to infect the lower female genital tract have been studied in an attempt to find a link with cervical neoplasia. Of the non-viral agents, Trichomonas vaginalis and Chlamydia trachomatis have been studied recently. A twofold increased incidence of antibodies against Chlamydia trachomatis was found in women with CIN when compared with controls.

However, most research interest has concentrated upon viral agents. Both Epstein-Barr virus and cytomegalovirus are thought to infect the cervix, but neither has been linked to the development of cervical neoplasia. Early work on herpes simplex virus type 2 (HSV-2) did demonstrate a possible link with cervical neoplasia. In the laboratory HSV-2 has been shown to be a very weak oncogenic virus; however, analysis of many cervical tumours has failed to show any evidence of HSV-2 deoxy ribo nucleic acid (DNA). Despite early case-controlled studies linking previous infection with HSV-2 to cervical neoplasia, large population studies have shown that the presence of HSV-2 antibodies is related to sexual activity rather than cervical neoplasia.[33]

Molecular Biology

In 1995, The World Health Organization (WHO) declared HPV as carcinogenic in humans from the biologic and epidemiologic points of view. There is strong evidence to support the role of HPV pathogenesis in cervical dysplasias and cervical cancer.[34] HPV DNA has been detected in greater than 90% cervical cancer tissues.

The HPV genome encodes for the following proteins.[35]

- 6E (early) proteins – Gene regulation and cell transformation
- 2L (late) proteins – Shell of the virus.
- 1 region – Regulation of DNA sequences

The HPV proteins E6 and E7 are important viral oncogenes involved in the pathogenesis of cervical cancer. These oncogenes have transforming properties that interact with growth regulating proteins. Their continued

expression is necessary for malignant transformation. It is important to point out that there are different levels of oncogenicity of E6 and E7, depending on their base pairs. E7 has more oncogenic properties on HPV 16 than on HPV 6.

The molecular processes by which these proteins cause malignant transformation involve the p53 and retinoblastoma systems (Rb). Normally p53 is a negative regulator of cell growth. When there is chromosomal damage, p53 allows DNA repair enzymes to function. The E6 protein binds and degrades p53, thus allowing the accumulation of mutations without DNA repair and thereby causing "unchecked" cellular cycling.[36] Apoptosis is therefore inhibited.

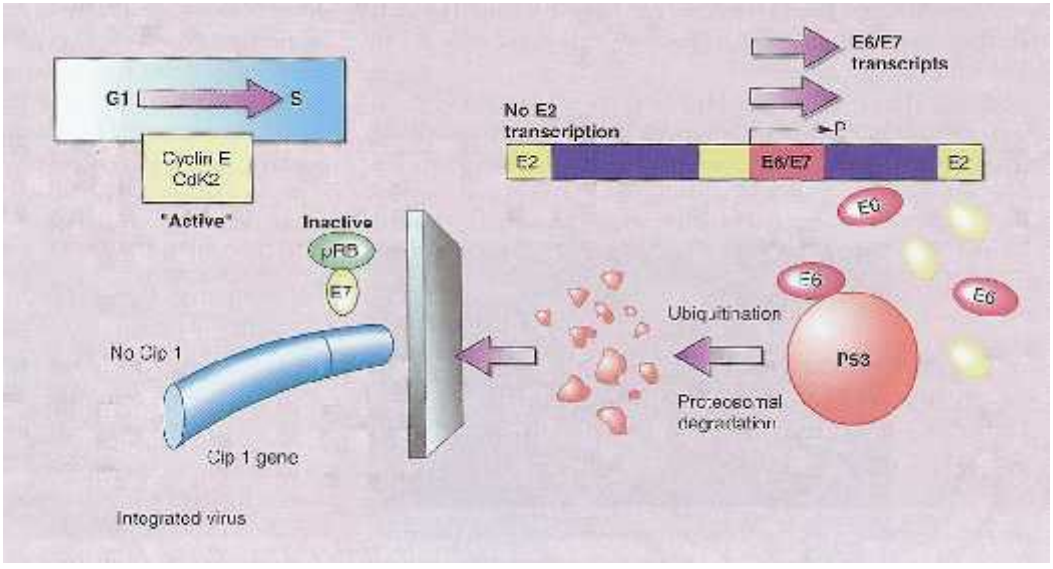


Figure 4: Hypothetical mechanism for cervical cancer tumorigenesis by the human papillomavirus

The Rb protein is involved in inducing cell apoptosis in response to DNA damage.[37] Rb binds to E2F transcription factor and inactivates E2F, which is involved with promoting the synthesis phase (S phase) of cell division. E7 bind to the Rb protein, which causes E2F to be released and allows cell division and

bypasses the "check" system with p53. E6 and E7 are also involved in apoptotic inhibition and cell transformation through other mechanisms.

The basic segments of the natural history of transient HPV infection include viral entry, viral replication, productive viral infection, and host containment. The viral life cycle is depicted in Figure 5.[28]

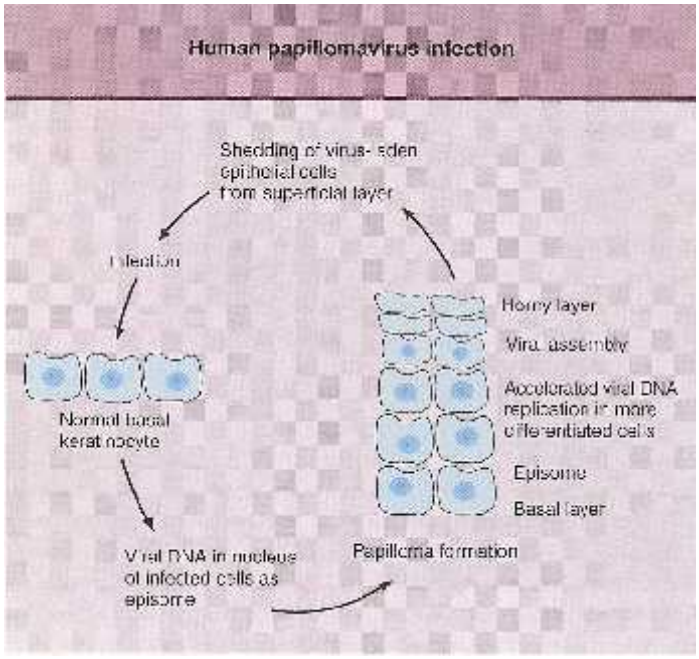


Figure 5: Human papillomavirus infection

Recent molecular studies have implicated specific genes that may be important in cervical tumorigenesis. Studies suggesting alterations of specific oncogenes in cervical tumorigenesis are summarized in Table 1. These findings are preliminary. However, they provide considerable evidence supporting the involvement of oncogene activation in the development of cervical carcinomas.

As mentioned earlier, losses of specific chromosomal regions, also known as allelic losses or LOH, are thought to target tumor suppressor genes.[27] Studies

of cervical carcinoma have described a high frequency of LOH of the short arm of chromosome 3[38] and the short arm of 17,[39] thus suggesting the possibility of suppressor genes in these regions. More extensive allelic loss studies are likely to implicate involvement of additional tumor suppressor genes.

Table 1: Summary of Oncogene Abnormalities in Cervical Carcinoma

Oncogene	Genetic Alteration	Summary of Results
ras Group	Over expression of p21	Increased staining by immunohistochemistry in CIN, invasive squamous carcinoma[40,41]
c-HA-ras	Somatic deletion/codon 12 mutation	Loss of one allele in 36% of informative cases, mutations in 24% of advanced – stage carcinomas[39]
c-myc	Over expression	Increased staining by immunohistochemistry in 50% of invasive carcinomas, over expression correlated with poor prognosis[42]
c-myc	Over expression	Increased staining by immunohistochemistry in 2/7 high-grade CIN and in 2/4 invasive carcinomas[43]
c-myc	Amplification	Amplification without structural gene rearrangement in 32% of invasive carcinomas[44]
c-myc	Amplification or gene rearrangement	Amplification and/or gene rearrangement in nearly 90% of invasive carcionomas[45]

PATHOGENESIS OF INVASIVE CERVICAL CANCER

Persistence of DNA from a high-risk type of HPV is significantly associated with development of high-grade CIN or dysplasia and invasive cervical cancer.[46,47] CIN III and invasive cancer are most commonly found at the junction between the squamous and glandular cells of the uterine cervix, which is called the squamocolumnar junction (SCJ). This cervical transformation zone, an active area of squamous metaplasia, is especially vulnerable to HPV-induced cellular transformation. After infection with HPV, one of two clinical pathways may occur;

1) Transient viral expression followed by clinical clearance

2) Viral persistence.

Most infections remain undetectable or produce transient cytologic changes that are missed by infrequent screening. Some infections cause detectable cytologic changes diagnostic of HPV infection, including a low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL), or induce the shedding of cells that demonstrate only some, but not all, features of an HPV infection (for example atypical squamous cells of undetermined significance (ASC-US). CIN is a tissue-based classification that is based on reviewing the histopathology of biopsy specimens. The designation of CIN I, II or III is based on the thickness of the epithelium showing abnormal cells, the degree of mitotic activity, and nuclear atypia as shown in Figure 6.[48]

Since more than one naming system is used, it is important to note that CIN I and II correspond to mild and moderate dysplasia, respectively; CIN III is analogous to severe dysplasia, and this category includes carcinoma in situ. Pathologists often disagree about the interpretation of samples at the lower end of the cytologic and histologic spectra (ASC-US and CIN I, respectively).[49] The disparity between the high rate of infection with HPV and the relatively low risk of cervical cancer reflects the fact that many women become infected with HPV but most infections spontaneously regress over time. Attempts to understand why the virus persists in some women have focused on variants of high-risk HPV types and cofactors such as smoking, other infectious agents (Chlamydia, herpes, bacterial vaginosis), parity, use of oral contraceptives, and host, immune factors. In the end, any important role played by HPV variants and cofactors is likely to be due to promotion of viral persistence because it is the most important factor in determining whether an HPV infection has the potential to progress to a precancerous lesion or cancer. The incidence of invasive cervical cancer reaches a plateau in women approximately 15 years later than the peak incidence of CIN III, suggesting a slow progression from CIN III to invasive carcinoma in most instances.[50] This prolonged period from development of CIN III to invasion probably reflects the long period of time required for the accumulation of mutations following viral integration and down-regulation of the apoptotic functions of certain tumor suppressor oncogenes (p53 tumor suppressor gene, or p53 and the retinoblastoma protein or pRb). Inhibition of p53 and pRb leads to an

increased cell proliferation rate and to genomic instability. The infected cell acquires more and more DNA damage that cannot be repaired leading eventually to oncogenic transformation.[28]

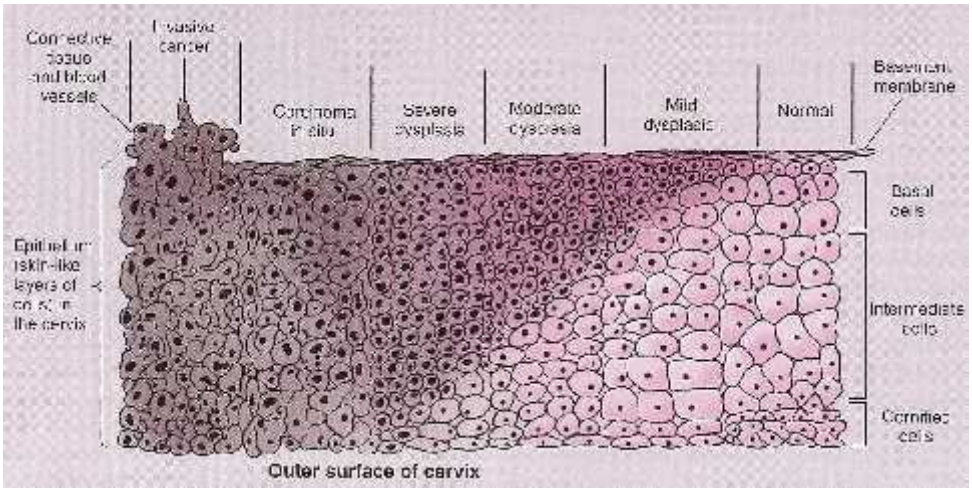


Figure 6: Schematic development of cervical cancer from normal and precursor lesions

The various primary malignant neoplasms of the uterine cervix are listed as below. The first two categories, squamous cell carcinoma and adenocarcinoma, constitute the vast majority of cervical carcinomas. The adenocarcinomas are, however, a rather complex and heterogeneous group whilst in recent years it has become increasingly clear that mixed carcinomas, which must be distinguished form double or collision tumors, may not be as uncommon as previously thought. The use of the term 'neoplasms of reserve cell origin[51] to describe these mixed tumors is probably a correct histogenetic categorization but there seems to be little justification for limiting the term to the mixed neoplasms when it is almost certain that the vast majority of carcinomas probably originate form these pluripotential cells. The undifferentiated carcinomas include a variety

of neoplastic entities which cannot currently be identified or separated by conventional light microscopy, electron-microscopy or immunohistochemistry. Primary malignant melanomas and non-epithelial tumors in this site are extremely rare.[30]

The conventional view of primary, overtly invasive epithelial tumors of the uterine cervix is that between 90 and 95% are squamous cell carcinomas and that adenocarcinomas make up the bulk of the residue. This view is now known to be incorrect for, in reality, only about 70%, at most, of cervical carcinomas are purely squamous whilst approximately 15% are adenocarciomas and eight to 10% are adenosquamous carcinomas.[30]

Malignant tumors of the uterine cervix[30]

Squamous carcinoma

- Well differentiated, keratinizing
- Moderately well differentiated, focally keratinizing
- Poorly differentiated,
    - Large cell, non-keratinizing
    - Small cell, non-keratinizing
- Verrucous carcinoma
- Papillary squamous carcinoma

Adenocarcinoma

- Endocervical adenocarcinoma
    - Minimal deviation adenocarcinoma (Adenoma malignum)
- Papillary serous adenocarcinoma

- Endometrioid adenocarcinoma
- Clear cell adenocarcinoma
- Mesonephric adenocarcinoma
- Enteric adenocarcinoma

Mixed tumors

- Adenosquamous carcinoma
    - Glassy cell carcinoma
- Adenoid cystic carcinoma

Small cell carcinomas

- Neuroendocrine tumors
- Adenoid basal carcinoma
- Subcolumnar reserve cell carcinoma

Undifferentiated carcinoma

Metastatic carcinoma

Malignant melanoma

Malignant non-epithelial tumors

- Sarcoma
- Lymphoma

FIGO STAGING SYSTEM FOR CERVICAL CANCER

The new classification and staging of cervical cancer adopted by the International Federation of Obstetrics and gynecology came into operation on Jan 1st 1962.[16]

FIGO staging system for cervical cancer[28]

Stage	Qualification
0	Carcinoma in situ, intraepithelial carcinoma. Cases of stage 0 should not be included in any therapeutic statistics for invasive carcinoma.
I	The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded)
IA	Invasive cancer identified only microscopically. All gross lesions, even with superficial invasion, are stage IB cancers. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and no wider than 7 mm. (The depth of invasion should not be more that 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates) vascular space involvement, either venous or lymphatic should not alter the staging.
IA1	Measured invasion of stroma no greater than 3 mm in depth and no wider than 7 mm.
IA2	Measured invasion of stroma 3 to 5 mm in depth and no wider than 7 mm.
IB	Clinical lesions confined to the cervix or preclinical lesions greater than IA.
IB1	Clinical lesions no greater than 4 mm in size.
IB2	Clinical lesions greater than 4 mm in size.
II	The carcinoma extends beyond the cervix but has not extended onto the pelvic wall; the carcinoma involves the vagina but not as far as the lower third.
IIA	No obvious parametrial involvement
IIB	Obvious parametrial involvement.
III	The carcinoma has extended onto the pelvic wall; on rectal examination there is no cancer free space between the tumor and the pelvic wall. The tumor involves the lower third of the vagina. All cases with a hydronephrosis or non functioning kidney should be included, unless they are known to be due to other cause.
IIIA	No extension onto the pelvic wall, but involvement of the lower third of the vagina.
IIIB	Extension onto the pelvic wall or hydronephrosis or non functioning kidney.
IV	The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum.
IVA	Spread of the growth of adjacent organs.
IVB	Spread to distant organs.

DIAGNOSIS OF CERVICAL CANCER

The common methods used to detect cervical cancer are; Obstetric examination

- Cytology
- Speculoscopy
- Microcalpohysterescope
- Calposcopy
- Punch biopsy
- Endocervical curettage
- Cone biopsy[52]

The following diagnostic aids are acceptable for determining a staging classification: physical examination, routine radiographs, colposcopy, cystoscopy, proctosigmoidoscopy, intravenous pyelogram (IVP) and barium studies of the lower colon and rectum. Other examinations, such as lymphography, computed tomography (CT) scans, magnetic resonance imaging (MRI) examinations, arteriography, venography, laparoscopy and hysteroscopy are not recommended for staging, since they are not uniformly available from institution to institution. Findings uncovered by CT scan or MRI examination can be utilized in the planning of therapy but should not influence the initial clinical staging of the lesion.[5]

Biochemical assays

Studies on tumor markers in cervical cancer

A heterologous antiserum for squamous cell carcinoma of the cervix was first reported in 1977 and designated tumour antigen 4 (TA-4). More recently, a purified fraction has been used – squamous cell carcinoma antigen. It is evident that this antigen is a useful marker in assisting clinical diagnosis and in follow-up after therapy.

A study of the distribution of antibody binding sites showed that anti-SCCA recognizes metaplastic, dysplastic and neoplastic squamous epithelium.[53] It has also been detected in a long established cervical cancer cell line, but not in cell lines derived from choriocarcinoma or breast carcinoma.[54]

In a population of normal subjects and those with benign and malignant gynaecological disease, SCCA showed useful marker properties. Of the non cancer, 4% showed raised levels, compared with 14% of those with CIN lesions and 67% of those with invasive lesions.[55] There was a good correlation with disease status following treatment. Similar results were reported in a more recent study.[56] The overall sensitivity rate was proportional to tumor differentiation (good 78%; poor 38%).

In comparison study with CA125 and carcinoembryonic antigen(CEA) in subjects with cervical adenocarcinoma, SCCA was superior to CEA but less useful overall than CA125.[57] In another study, with predominantly squamous cell leions,[58] SCCA was superior to CA125 and CEA. The median lead time for

SCCA to detection of clinical recurrence was 13 months (range two to 52 months). Comparison analysis are as given below.

Marker	Result
SCCA, CA125, CEA	SCCA paralleled disease state, not others.[58]
SCCA, CA125, CEA	CA125 superior sensitivity and disease correlation in surgical stage adenocarcinoma (Including adenosquamous cell carcinoma)[57]
CA125, CEA, CA19-9	CEA and / or CA19-9 high CA 125 low in adenoca; reverse with normal / benign CEA + CA19 – 9 / CA125 ratio useful.[59]

Enzyme screening test

When normal cells undergo dysplasia and progress to stage 0 and invasive cancer, glycogen metabolism is disturbed. This is associated with chromatin and chromosomal changes and with the increased nucleotide needs of proliferating cells. Bonham (1964) estimated the activity in vaginal and uterine fluid of 6-phosphogluconate dehydrogenase (6 PGD). This is an enzyme of the pentose phosphate pathway from glycogen to nucleotides which is active only in the presence of nicotinamide adenine dinucleotide phosphate (NADP+), magnesium and certain other ions.[16]

Role of enzyme markers in cervical cancer

Enzymes are present in much higher concentrations inside the cell. Enzymes are released into the systemic circulation as the result of tumor necrosis or a change in the membrane permeability of the cancer cells. Elevated enzyme levels may signal the presence of malignancy.[11]

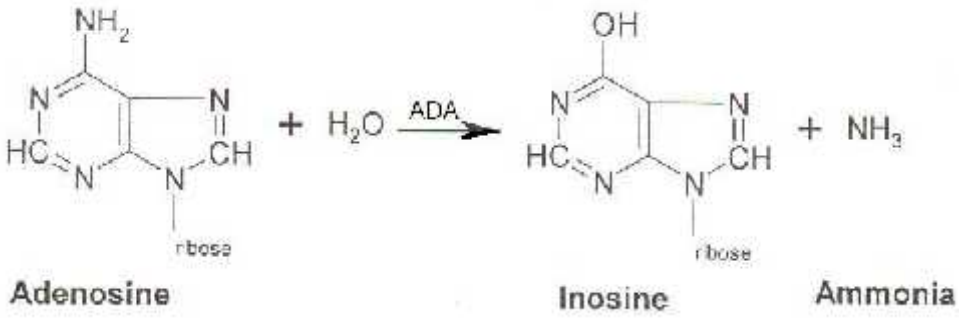
Leakage of enzymes from cells

Enzymes are retained within their cells of origin by the plasma membrane surrounding the cell. The plasma membrane is metabolically active part of the cell, and its integrity depends on the cell's production of adenosine triphosphate (ATP). Any process that impairs ATP production, either by depriving the cell of oxidizable substrates or by reducing the efficiency of energy production by restricting the access of oxygen (Anoxia), promotes deterioration of the cell membrane.[60] The membrane becomes leaky and membrane constituents are shed into the surrounding milieu at increased rate when cells replicate more rapidly. Small molecules are the first to leak from damaged or dying cells, followed by larger molecules, such as enzymes. Cytosolic enzymes appear early in the serum, followed much later by mitochondrial and membrane bound enzymes.[11]

ADENOSINE DEAMINASE: (Adenosine aminohydrolyse EC : 3.5.4.4)

Adenosine deaminase was identified by Spencer et al.[61] is involved in the catabolism of purine bases capable of catalyzing the deamination and hydrolytic cleavage of adenosine irreversibly converting it into inosine and ammonia.[62] The enzyme contains at least reactive thiol groups.[10]

It is widely distributed in human tissues, highest being in the lymphoid tissue. This enzyme is considered as an ectoenzyme.[63] Since it is found on the surface of many cells and is located in the cell membrane.[64]



Isoenzymes forms of ADA

Human ADA exists in at least three molecular forms ADA_1, ADA_{1+CP}, ADA_2.

ADA_1 - Spleen, lymphocytes, monocytes and neutrophils.

ADA_{1+CP} - Liver, lung, muscle, pancreas, kidney. (This is modified form of ADA_1).

ADA_2 - It could be detected only in monocytes.[65]

These different forms of ADA are separated by polyacrylamide gel electrophoresis.[66]

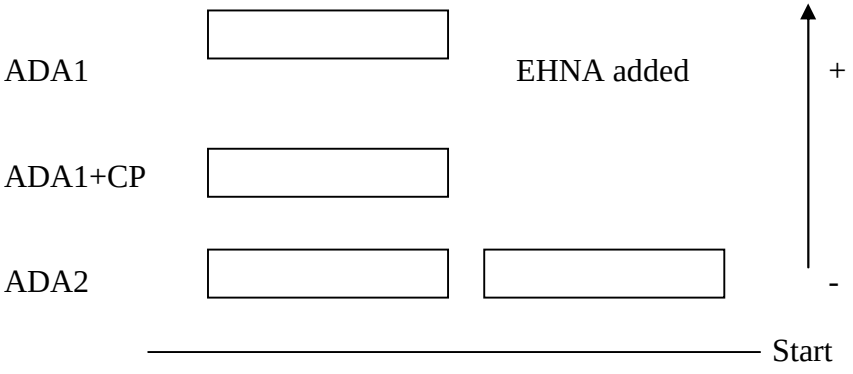


Figure 7: Separation of isoenzymes of ADA by electrophoresis

But only two principle isoenzymes are important.

- ADA_1 (ADA_1 & ADA_{1+CP}) and
- ADA_2

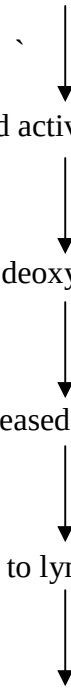
These have different optimal pH, Michaelis constant, relative specificity patterns and also gene located on different loci.[67] The structural gene for ADA is located on chromosome 20.[10] Locus containing 12 exons.[67]

ADA_1 has roughly equal affinities for adenosine and 2'deoxyadenosine and is found in many tissues.[68] Decreased ADA_1 in the lymphocytes and erythrocytes causes severe combined immunodeficiency syndrome (SCID). In this condition dysfunction of both B and T- lymphocytes with impaired cellular immunity and decreased production of immunoglobulins. ADA deficiency accounts for about 50% of causes of autosomal recessive SCID.[69]

Molecular defect

Specific point mutation in Exon 11 accounts for a high proportion of these, while a few mutations are homozygous for large deletions encompassing Exon 1.

Most of the mutations in the gene to ADA so far detected have been base substitutions, though deletions have also been detected.



Reduced activity of ADA

Accumulation of deoxyadenosine and dATP

Increased toxicity

Injury to lymphocytes

Decrease in cell mediated and humoral immunity[69]

ADA_2 is the major component of the activity of total ADA in the serum of healthy persons. ADA_2 has much greater affinity for adenosine and is found only in macrophages and monocytes, which release it when stimulated in the presence of live organisms.

Tissue distribution of ADA

The study of distribution of ADA in various organs of rabbit was first studied by Convey and Cooke in 1939.[70] ADA distribution in humans is ubiquitous.[61]

In humans the highest activity of ADA is found in thymus, lymphoid tissues (~800IU/mg) and the lowest in erythrocytes (~1 IU.mg).

Among non-lymphoid tissues in humans relatively high levels of ADA is found in the villi of epithelial cells lining the duodenum (570 IU/mg); levels are lower in the other portion of the GI tract. Tissues such as muscle, lung, liver, kidney, brain and blood have low activity in most species.[68]

ADA activity

The bulk of the activity is localized in the cytosol of the lymphocytes.[71] The activity is 10 times greater in lymphocyte than erythrocytes.[72] ADA activity in T lymphocytes is five to 20 times higher than B-lymphocytes,[73] as its activity is related to the differentiation of lymphnodes.[74]

Functions of ADA

1) This enzyme deaminates adenosine, deoxyadenosine and certain synthetic ribosides.[10]

2) ADA in purine salvage pathway: ADA catalyzes the irreversible hydrolytic deamination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively (Figure 8).[75]

3) The physiological roles of ADA can be seen in connection with adenosine, the concentration of which can be modulated by enzymatic action of ADA. An increase in adenosine leads to coronary vasodilation, reduction in heart rate and contractile force, inhibition of platelet aggregation, mast cell granulation, inactivation of eosinophil migration, renal vasoconstriction, regulation of ion channel activity, membrane potential and release of neurotransmitter hormone.[76]

4) ADA is essential for differentiation and proliferation of lymphoid cells particularly T-cells.[74]

5) ADA plays role in maturation of monocytes to macrophages.[77]

6) ADA activity is increased during cellular activation for energy demand to detoxify toxic metabolites.[62]

7) ADA for gene therapy: Adenosine deaminase deficiency has become important because it is the first disease to be treated by somatic gene therapy. One reason for selecting ADA deficiency as a suitable condition for somatic gene therapy was that cells that, express the gene for ADA, would have a selective advantage for growth over, uncorrected cells. The trial began in 1996 using retroviral medicine to transfer ADA gene into their T-cells. The number of T-cells normalized as did many cellular and humoral immune response. Gene treatment ended after 2 years, but integrated vector and ADA gene expression persisted and it was concluded that gene therapy can be safe and effective addition to the treatment of this condition.[69]

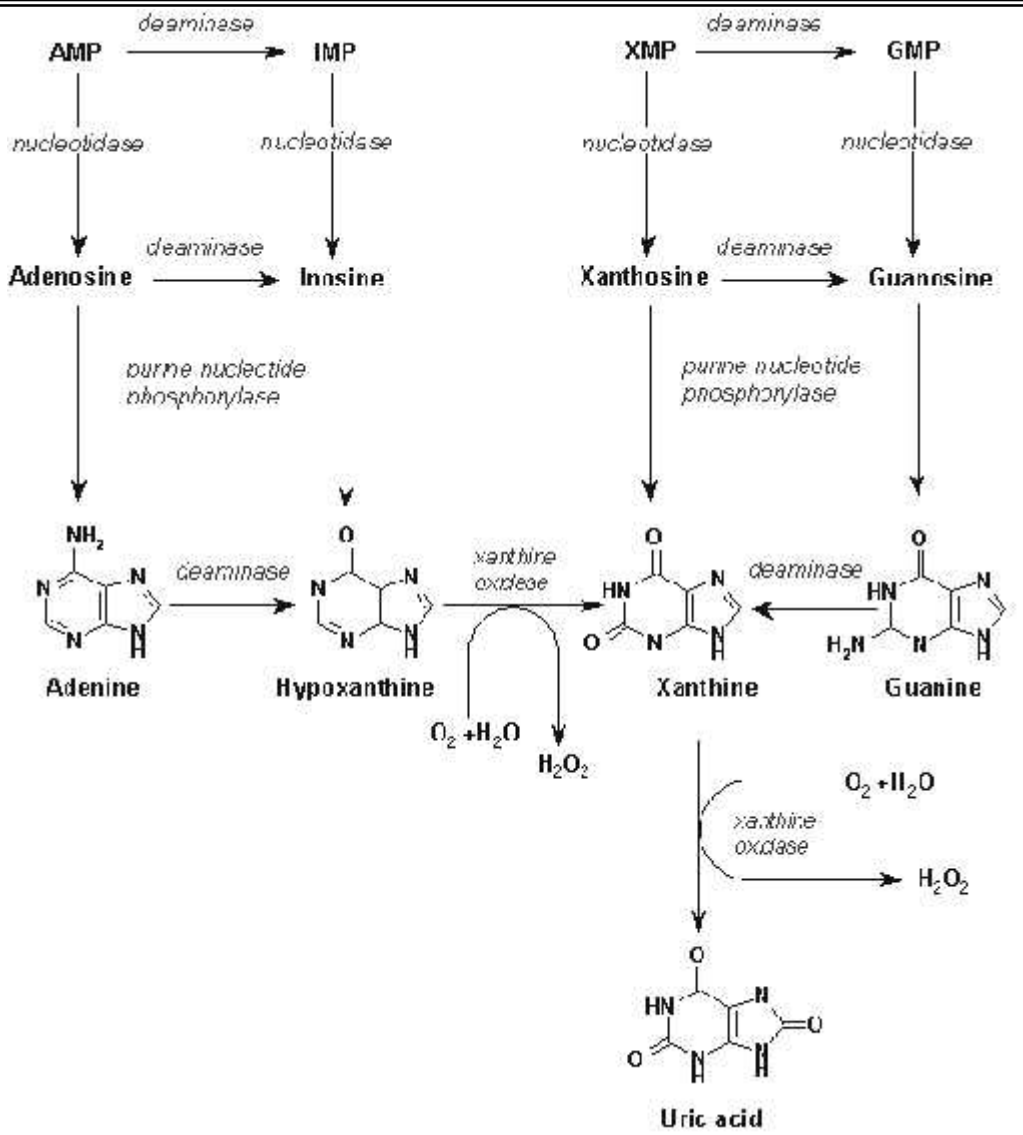


Figure 8: Purine salvage pathway

Role of serum ADA activity in cervical cancer

There are very few studies available on altered levels of ADA in cervical cancer. According to the study conducted by Borzenko BG, on the activity of adenosine and thymidine metabolism enzymes in serum of oncological patients they concluded that, the purine nucleoside adenosine is produced at increased levels in the tissues of solid cancers as a result of local hypoxia. Adenosine inhibits the cell mediated anti tumour immune response, promotes tumor cell migration and angiogenesis, and stimulates the proliferation of tumor cells. As a

result serum ADA activity is also increased to detoxify the high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues. It has also been suggested that increased ADA activity might be a physiologic attempt of the cancer cells to provide more substrates needed by cancer cells to accelerate the salvage pathway activity.[78]

In the study conducted by Specchia G and co investigators it was concluded that lymphocyte ADA activity in patients with gynecologic malignancies shows a broad range of variability. Among the gynecologic malignancies, they included one case with adenocarcinoma of the cervix and they inferred that ADA assessment in a larger series of cancer patients may add further prognostic information.[79]

Pratibha K and co-investigators studied on increased activity of ADA in acute infective hepatitis patients, and concluded that lipid peroxidation is followed by loss of structural integrity of plasma membranes. As a result, there occurs a release of membrane associated enzyme ADA into the circulation.[64]

The results of the study conducted by Naidu SK and co-researchers showed upsurged lipid peroxidation in cervical cancer patients, which is a consequence of increased free radical generation. It causes profound alterations in the function of the cell membrane and also structural organization of DNA leading to mutations. They concluded that lipid peroxidation is one of the possible cause of cervical cancer progression.[80] According to the some of the studies, lipid peroxidation was significantly increased in cervical cancer patients.[81,82,83,84]

GAMMA-GLUTAMYL TRANSFERASE (GGT): (E.C. 2.3.2.2)

(Gamma-glutamyl peptide: amino acid γ-glutamyl transferase E.C.2.3.2.2)

It is a peptidase that catalyzes the transfer of the γ-glutamyl group from a peptide gamma-glutamyl-peptide to an acceptor peptide or an L-amino acid. It is equally effective at removing the γ-glutamyl group from simpler compounds such as N- γ-glutamyl naphthylamide and N- γ-glutamyl-p-nitroanilide.[10]

$$\text{Glutathione + Amino acid} \xrightarrow{\text{GGT}} \text{glutamyl peptide + L-cysteinyl glycine}$$

It is a microsomal glycoprotein enzyme, first recognized by Hanes et al. in 1952. In most biological systems, glutathione serves as γ-glutamyl donor. This enzyme was originally termed as transpeptidase but the more appropriate term is transferase.

GGT in the serum has a molecular weight of 90,000 daltons, as measured by electrophoresis. The main fraction of normal serum migrates with the fast α2-globulins, while two minor bands of activity are found with the slow α2-globulins. The activity of the minor band is considerably increased in hepatitis and biliary obstruction, but the diagnostic value of GGT isoenzymes remains to be established.

Tissue distribution

The enzyme was first identified in kidney tissue where its concentration is very high but significant amounts occur in the liver and pancreas and only minor quantities in the intestine, heart and other tissues. It has thus proved useful in the investigation of disease of liver and biliary system.[60]

Functions of GGT

It has been suggested that GGT is involved in;

1) The transport of amino acids and peptides into the cell across the cell membrane in the form of γ-glutamyl peptides as the larger fraction of the enzyme is located in the cell membrane.

2) Peptide and protein biosynthesis.

3) Regulation of tissue glutathione levels and its metabolism.[11]

Clinical Significance

Even though renal tissue has the highest level of GGT, the enzyme present in serum appears to originate primarily from the hepatobiliary system and GGT activity is elevated in any and all forms of liver diseases.

In the liver GGT is located in the canaliculi of the hepatic cells and particularly in the epithelial cells lining the biliary ductules. In the liver cell the enzyme exists in two forms, a minor soluble form of low molecular weight in the cell, and a high molecular weight membrane bound microsomal component. Higher levels are generally observed in biliary tract obstruction. It is highest in cases of intrahepatic or post hepatic biliary obstruction, reaching levels five to 30 times normal. It is more sensitive than alkaline phosphatase (ALP), arylamidase and the transaminases in detection of obstructive jaundice, cholangitis and cholecystitis. The rise in GGT activity occurs earlier in these diseases than do these other enzymes and persists longer. Moderate elevations (two to five times normal) occur in infectious hepatitis.

Elevated levels of GGT are noted in the sera of individuals with alcoholic cirrhosis and in the majority of sera from those who drink heavily and in individuals receiving drugs such as phenytoin, Phenobarbital. The release of GGT into serum reflects the toxic effects of alcohol and other drugs on microsomal structures in liver cells, as within the hepatic parenchyma, GGT exists to a large extent in the smooth endoplasmic reticulum and is therefore subjected to hepatic microsomal induction. Hepatic complications occurring in cases of cystic fibrosis (mucoviscoidosis) also lead to elevations of GGT.

Patients with primary or secondary hepatic malignancy with jaundice almost invariably have raised GGT activities with levels averaging fifteen times the upper limit of normal.

GGT activity in serum can be elevated in some non-hepatic disorders such as acute pancreatitis, carcinoma head of the pancreas, congestive cardiac failure, myocardial infarction (MI) and diabetes mellitus (DM).

In cases of acute and chronic pancreatitis and in some pancreatic malignancies (especially those associated with hepatobiliary obstruction) enzyme activity may be five to 15 times the upper limit of normal. Normal levels of the enzyme are found in cases of skeletal disease (Paget's disease, bone neoplasms) children older than one year and healthy pregnant women all conditions in which ALP is elevated. Thus measurement of GGT levels in serum helps ascertain whether observed elevations of ALP are caused by skeletal disease or reflect the presence of hepatobiliary disease.

High levels of GGT are present in the prostate, which may account for the higher activity of GGT in sera of men, which is approximately 50% higher than in sera from women.[85]

Serum GGT activity in cervical cancer

Cytochemically detectable GGT activity appears whenever alterations of the normal epithelial microenvironment occurs. There are few studies available on altered levels of GGT activity in cervical cancer.

de Camargo JL and co-investigators studied GGT activity in cervical smears of patients with inflammatory or paraneoplatic or neoplastic conditions had GGT- positive cells. None of the normal cases showed any epithelial cells with GGT activity. They concluded that cytochemically detectable transpeptidase activity appears whenever alterations of the normal epithelial microenvironment occurs, but is not necessarily linked to the carcinogenic process. Therefore, cytochemically GGT positive cells should not be used as an indicator of neoplastic transformation of the cervical epithelium.[86]

Malkin A and co-investigators studied serum GGT along with CEA, pregnancy-associated macroglobulin (PAM) and PLAP in patients with ovarian and cervical cancer. The data showed the frequency of GGT positivity increased with more advanced disease in cervical cancer and GGT reflected tumor burden in carcinoma of the cervix.[14]

METHODOLOGY

The present study was conducted on patients of cervical cancer admitted at the KLES Dr. Prabhakar Kore Hospital and Medical Research Centre and Cancer Hospital, Belgaum during a period of one year.

Study design

Cross sectional study.

Study period

The present study was conducted during the period of May-2007 to May-2008.

Sample size

The present study comprised of 40 healthy females in the age group of 35-65 years as controls and 40 clinically and histopathologically confirmed patients of cervical cancer of the same age group. Cases were grouped into stage I, II and III.

Sample procedure

Sample size was calculated by taking 80% of the average of the previous three years.

Selection Criteria

Inclusion Criteria

- Clinically and histopathologically confirmed cases of cervical cancer.

Exclusion Criteria

Patients suffering from,

1. Myocardial infarction
2. Chronic obstructive pulmonary disease
3. Hepatocellular damage
4. Pancreatic disease
5. Renal failure
6. Diabetes mellitus
7. Tuberculosis
8. Immunocompromised status
9. Other malignancies
10. Alcoholics and smokers

PROCEDURE

All cases were evaluated and selected by simple random technique after fulfilling the selection criteria. The study was approved by the Ethical and Research Committee of J. N. Medical College, Belgaum.

Clinically and histopathologically confirmed cases of cervical cancer reported to Department of Obstetrics and Gynaecology at K.L.E.S. Dr. Prabhakar Kore Hospital and Medical Research centre and Cancer Hospital, Belgaum were

screened. After finding the suitability as per inclusion and exclusion criteria they were requested to participate in the study and briefed about the nature of the study, the interventions used and written informed consent was obtained (Annexure–I). The consented patients were enrolled in the present study. Further descriptive data of the participants like name, age, sex, detailed history, were obtained by interviewing the participants and recorded on predesigned and pretested proforma (Annexure-II).

Collection of Blood Samples

About five ml of venous blood was collected from antecubital vein under aseptic precautionary measures using sterile disposable syringe. The blood was allowed to clot and serum was separated by centrifugation and stored at $4^{\circ}C$. The estimation of parameters were carried out immediately. The following methods were used for the assay of enzymes :

1. Serum ADA : Galanti and Giusti Method.[87]
2. Serum GGT : Carboxy substrate method.[88]

ESTIMATION OF ADENOSINE DEAMINASE IN SERUM BY GALANTI AND GUISTI METHOD[87]

Principle

Adenosine deaminase hydrolyses adenosine to ammonia and to inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a

catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{hypochlorite} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Blue indophenol complex}$$

Reagents

The following reagents are supplied by the company Microxpress – A division of Tulip Diagnostics (P) Ltd., Goa.

a) ADA – MTB reagent (L1) : Buffer reagent, ready to sue.

b) ADA – MTB reagent (L2) : Adenosine reagent, ready to use.

c) ADA – MTB reagent (L3) : Phenol reagent

d) ADA – MTB reagent (L4) : Hypochlorite reagent

e) ADA – MTB standard (S) : ADA standard, ready to use.

Reagent preparation

Reagents L1, L2 and standard (S) are ready to use. Both the phenol reagent (L3) and hypochlorite reagent (L4) need to be diluted 1:5 with distilled water before use (1 part of reagent + 4 parts of distilled water).

Storage and Stability

1) Store the reagents at 2 to 8°C away from light when not in use.

2) Stability of the reagents is as per the expiry date mentioned on the label.

3) The working phenol reagent and working hypochlorite reagent are stable for 6 months when stored at 2 to 8˚C in tightly closed bottles.
4) ADA is reported to be stable in serum for three days at 2 to 8˚C as after this, ammonia may be released in the samples even without any microbial contamination.

Conditions to be maintained to get accurate results

1) Adenosine reagent may form crystals at 2 to 8˚C. Dissolve the same gently by warming the reagent for sometime before use at 37˚C to 50˚C.
2) The kit components form the same lot should be used for achieving accurate and reproducible results. Do not intermix reagents from different lots.
3) The sequence of addition of reagents should be followed meticulously.
4) Water bath temperature should be maintained at 37˚C.
5) Do not use haemolyzed, contaminated or turbid sample specimens.

Test Procedure

1. Bring all regents and samples to room temperature before use.
2. Prepare the working phenol reagent and working hypochlorite reagent.
3. Set the spectrophotometer filter at 570-630 nm (Hg 578 or 623 nm) at 37˚C.
4. Pipette into clean dry test tubes labeled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows:

Additional Sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Buffer reagent	0.20	0.20	--	--
Adenosine reagent	--	--	0.20	0.20
Deionised water	0.02	--	--	--
Standard	--	0.02	--	--
Sample	--	--	-	0.02

5. Mix well and incubate at 37˚C for exactly 60 minutes and then add the following.

Additional Sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Working phenol reagent	1.00	1.00	1.00	1.00
Sample	--	--	0.02	--
Working hypochlorite reagent	1.00	1.00	1.00	1.00

6. Mix well and incubate at 37˚C for 15 minutes or at room temperature for 30 minutes.

7. Measure the absorbance of the blank (Abs. B), standard (Abs. S), sample (Abs. SB) and test (Abs. T) against distilled water.

Calculation

$$\text{Total ADA activity in U/L} = \frac{\text{Abs. T} - \text{Abs. SB}}{\text{Abs.S} - \text{Abs.B}} \quad \text{X } 50$$

Reference Values

Sample	Prediction	Values
Serum, Plasma, Pleural, Pericardial and Ascitic Fluids	Normal Suspect Strong Suspect Positive	<30 U/L 30 U/L to 40U/L > 40 U/L to 60U/L > 60U/L
CSF	Normal Positive	< 10U/L > 10U/L

ESTIMATION OF GAMMA GLUTAMYL TRANSFERASE IN SERUM BY CARBOXY SUBSTRATE METHOD[88]

Principle

Gamma glutamyl transferase catalyzes the transfer of amino group between L-γ-Glutamyl-3-carboxy-4 nitroanilide and glycylglycine to form L-γ-glutamylglycyl glycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity in the sample.

L-γ-Glutamyl-3-Carboxy-4nitroanilide + Glycylglycine $\xrightarrow{\text{GGT}}$ L-γ-Glutamylglycylglycine + 5-amino-2-nitrobenzoate

Reagents

The following reagents supplied by the company Crest Biosystems – A division of Coral Clinical Systems, Goa.

L1: Buffer reagent

T1: Substrate tablets.

Reagents preparation

Working reagent is prepared by dissolving one substrate tablet in 2.2 ml of buffer reagent.

Reagent storage and stability

1) Store reagent at 2 to 8˚C.
2) Stability of the reagent is as per the expiry date mentioned on the label.
3) Working reagent is stable for at least 15 days when stored at 2 to 8˚C.
4) Storage of specimen – Activity of serum GGT is reportedly stable for at least eight hours at 25˚C, three days at 2 to 8˚C or one week at -20◦C
5) The reagent should not be used if moisture has entered the vial.

Procedure

1. Bring all reagents and samples to room temperature before use.
2. Set the spectrophotometer to zero with distilled water at 405nm.
3. Set cuvette temperature at 37˚C.
4. Pipette 1ml of working reagent in clean dry test tube.
5. Incubate at the assay temperature for one minute and add 0.1 ml of serum to regent, mix and immediately transfer to cuvette.
6. Mix well and read the initial absorbance A_0 after one minute and repeat the absorbance reading after every one, two and three minutes.
7. Calculate the mean absorbance change per minute (ΔA/min).
8. Multiply the ΔA/min by the factor 1158.

Calculation

GGT Activity in U/L = ΔA/min X 1158

Reference values

Serum

Males: 10 to 50 U/L at 37˚C.

Females : 7 to 35 U/L at 37˚C.

Statistical analysis

Statistical analysis of all the obtained parameters in patients with cervical cancer and control groups were done using student's 't' test. The mean and standard deviation (S.D.) for each of the outcome was computed. The comparison between controls and various stages of cervical cancer was done by analysis of variance (ANOVA) followed by Scheffe HSD multiple comparison test.

RESULTS

The present study comprises of 80 participants, 40 healthy controls and 40 cases of cervical cancer, which was confirmed by clinical and histopathological examination and the mean age is matched in two groups. The findings are tabulated as below.

Table No. 2: Mean age

Group	Mean Age	Standard deviation
Cases (n=40)	48.45	8.69
Control (n=40)	48.63	8.59

t = 0.091 df = 78 p = 0.928

The mean age for controls was 48.63 ± 8.59 years and for cases 48.45 ± 8.69 years, with p value of 0.928. Hence age is matched in the two groups.

Table No. 3: Age distribution according to stages of cervical cancer

Stages	Mean Age	Standard deviation
Stage I (n=5)	46.80	9.20
Stage II (n=9)	48.33	9.96
Stage III (n =26)	48.81	8.47
Controls (n=40)	48.63	8.59

F value for 3, 76 = 0.077 p = 0.972

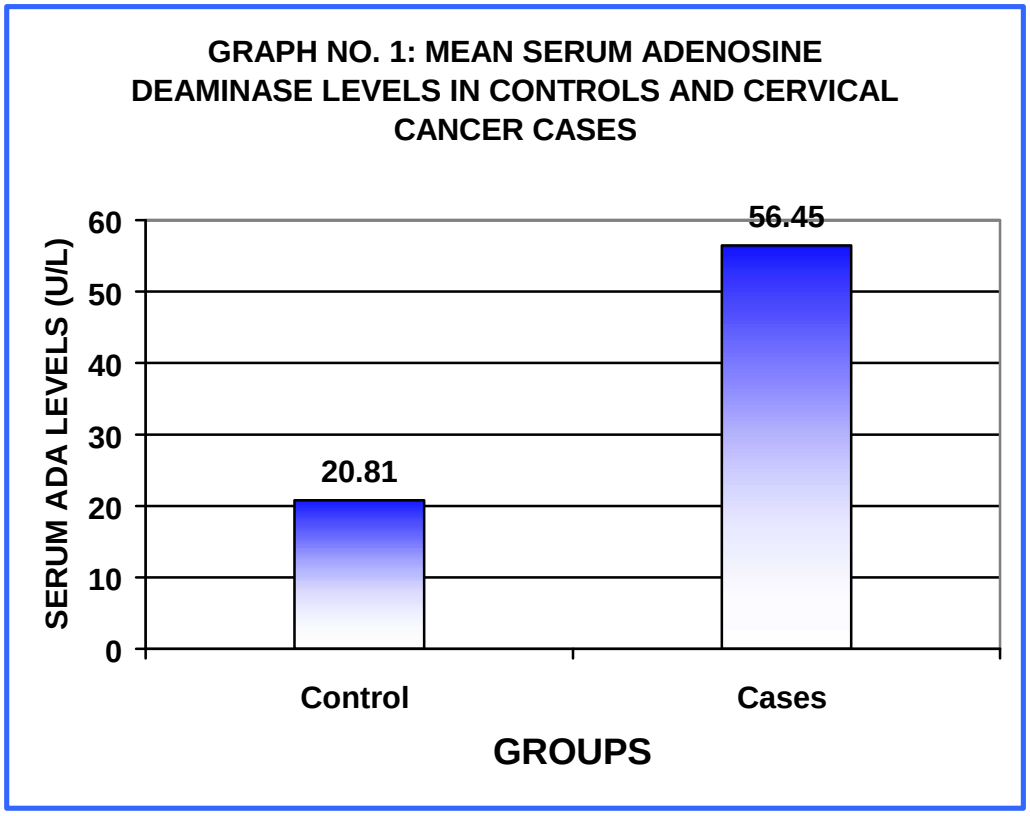
The mean age for controls was 48.63 ± 8.59 years, stage I 46.80 ± 9.20 years, stage II 48.33 ± 9.96 years and stage III 48.81 ± 8.47 years with a p value of 0.972. Hence there was no difference in the mean age of cases compared with controls.

Table No. 4: Serum enzyme levels in controls and cervical cancer cases

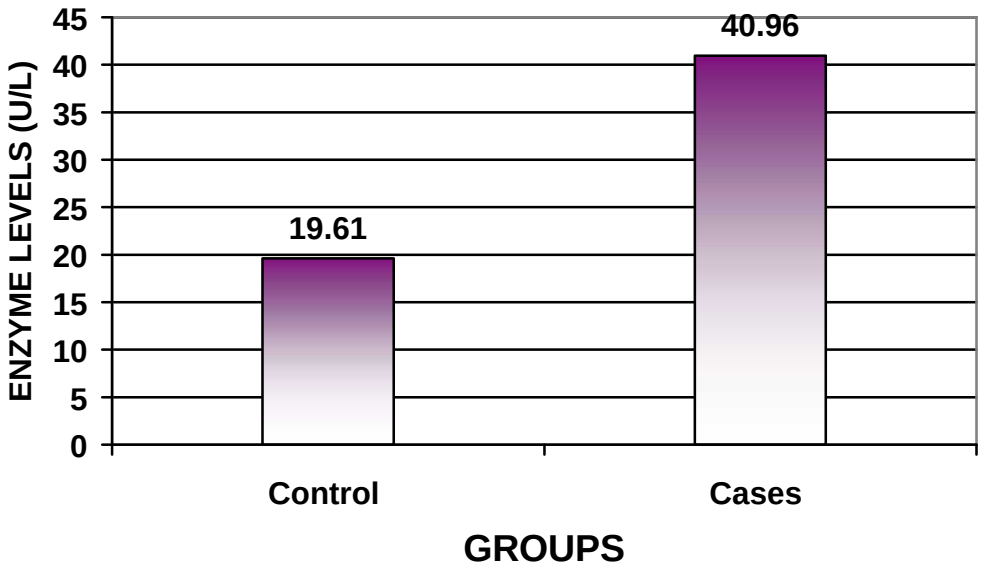
Enzymes	Control		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
ADA (U/L)	20.81	5.97	56.45	19	< 0.001
GGT (U/L)	19.61	5.80	40.96	8.45	< 0.001

For ADA F value for 3, 76 = 47.531

For GGT F value for 3, 76 = 64.206



GRAPH NO 2: MEAN SERUM GAMMA GLUTAMYL TRANSFERASE LEVELS IN CONTROLS AND CERVICAL CANCER CASES



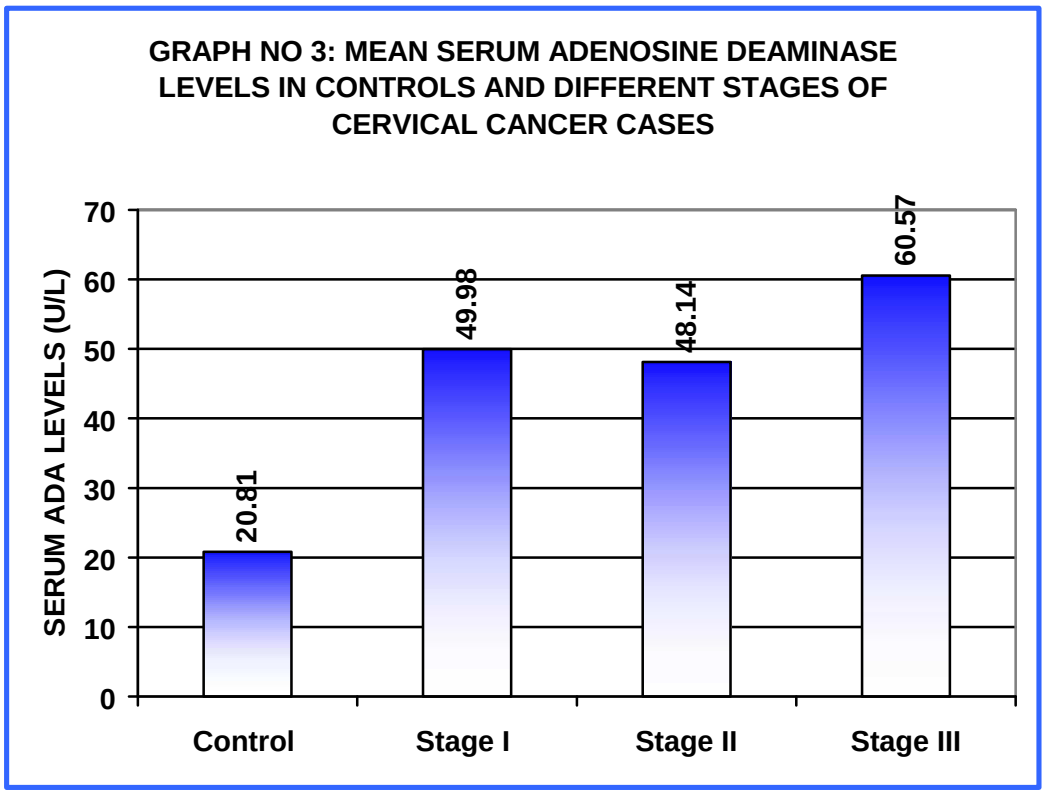
In the present study, mean serum adenosine deaminase level in controls was 20.81 ± 5.97 U/L while in the patients of cervical cancer it was 56.45 ± 19 U/L. A highly significant increase in serum ADA was observed in cervical cancer patients as compared to controls with p value less than 0.001 as shown in Table No 4 and Graph No. 1.

The mean serum Gamma glutamyl transferase level was 19.61 ± 5.80 U/L in controls whereas in cervical cancer patients it was 40.96 ± 8.45 U/L. A significant increase in GGT was observed in cervical cancer patients when compared to controls with a p-value < 0.001. (Table No 4 and Graph 2).

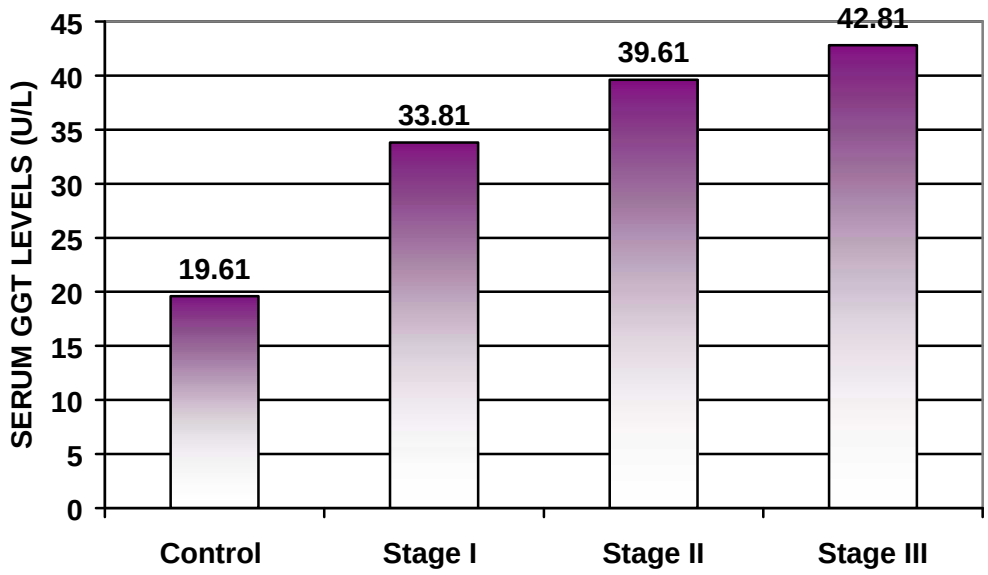
Table No. 5. Serum enzyme levels in controls and different stages of cervical cancer patients

Serum Enzymes	Control		Stage I		Stage II		Stage III	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
ADA (U/L)	20.81	5.97	49.98*	23.56	48.14*	5.56	60.57*	20.35
GGT (U/L)	19.61	5.80	33.81*	5.20	39.61*	11.48	42.81*	7.10

* $p < 0.001$ statistically significant



GRAPH NO 4: MEAN SERUM GAMMA GLUTAMYL TRANSFERASE LEVELS IN CONTROLS AND DIFFERENT STAGES OF CERVICAL CANCER CASES



Serum ADA levels at the various stages of cervical cancer Stage I (49.98 ± 23.56 U/L), Stage II (48.14 ± 5.56 U/L) and stage III (60.57 ± 20.35 U/L) was also estimated and compared with controls (20.81 ± 5.97 U/L). A significant increase in serum ADA was observed in Stage I, II and III when compared to controls with p value of < 0.001 (Table No 5 and Graph 3).

Serum GGT levels at the various stages of cervical cancer Stage I (33.81 ± 5.20 U/L), Stage II (39.61 ± 11.48 U/L) and stage III (42.81 ± 7.10 U/L) was also estimated and compared with controls (19.61 ± 5.80 U/L). A significant increase in serum GGT was observed in Stage I, II and III when compared to controls with p value of < 0.001 (Table No 5 and Graph 4).

In the present study an interstage comparison of serum ADA levels showed a non significant increase in levels of ADA, compared between stage I

and Stage II with P value of 0.995, Stage I and Stage III with P value of 0.393 and between stage II and III with p value of 0.096.

An interstage comparison of serum GGT levels showed a non-significant increase in levels of GGT, compared between stage I and stage II with p value of 0.452. stage II and stage III with p value of 0.642. While moderately significant increase in stage I and stage III with p value of 0.050.

DISCUSSION

When we think of cancer in general terms, we are apt to conjure up a process characterized by a steady, remorseless and inexorable progress in which the disease is all conquering and none of the immunological and other defensive forces will help leading to faltering footsteps to the grave.

Worldwide, invasive cervical cancer is the most common genital female malignancy and the second most common malignancy in women, after breast cancer. As there is no population based screening programme in India, 70 to 80% of the cervical cancer patients are diagnosed at advanced stages with very poor long term survival. Surgery, radiation or chemoradiation therapies are the only available treatment for cancer cervix, are not accessible to all the victims in the developing countries and when available, women suffer from severe morbidities with overall survival rate of 40%. Hence prevention is the most primary approach to control this disease.

Early detection of cancer offers the best chance for cure. Tumor markers used for screening, diagnosis, staging, prognosis and to monitor treatment of cervical cancer are expensive and analytical method for determination of these need well established centres and are expensive. As there are very few studies on enzyme markers in cervical cancer the present study has been undertaken to assess the reliability of some of the enzyme markers namely, serum adenosine deaminase and gamma glutamyl transferase which are inexpensive, rapid, analysed by easy methods and may be used as supportive parameters for diagnostic purpose and may add further for prognostic information.

In the present study, a significant increase in serum adenosine deaminase activity was observed in cervical cancer patients as compared to controls. An interstage comparison showed a non significant increase in serum ADA level from stage I to stage III. The study results are in accordance with the findings of Borzenko BG, who studied on the activity of adenosine and thymidine metabolism enzymes in the serum of oncological patients. They concluded that, purine nucleoside adenosine diaminase produced at increased levels in the tissues of solid cancers as a result of local hypoxia. Adenosine inhibits the cell mediated anti tumour immune response, promotes tumor cell migration and angiogenesis and stimulates the proliferation of tumor cells. As a result, serum ADA activity is also increased to detoxify high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues.[78]

Speechia G and co investigators reported that lymphocyte ADA activity in patients with gynaecologic malignancies shows a broad range of variability.[79]

The results of the study conducted by Naidu SK and co-researchers showed upserged lipid peroxidation in cervical cancer patients which is consequence of increased free radical generation. It causes profound alterations in the function of the cell membrane and also structural organization of DNA leading to mutations.[80] In accordance to this, some of the studies showed, lipid peroxidation was significantly increased in cervical cancer patients.[81,82,83,84]

Pratibha K and co-investigators studied on increased activity of ADA in acute infective hepatitis and concluded that lipid peroxidation is followed by loss

of structural integrity of plasma membranes. As a result, there occurs a release of membrane associated enzyme ADA into the circulation.[64] Measurement of malondialdehyde (MDA) in this study would have been useful in stating that lipid peroxidation is one of the possible cause of cervical cancer progression. From the other studies we can co-relate with increased lipid peroxidation in cervical cancer patients[81,82,83,84] and release of membrane associated enzyme ADA into the circulation.

According to our study, serum gamma glutamyl transferase level was significantly increased in cervical cancer patients when compared to controls. An interstage comparison showed a non-significant increase in serum GGT level from stage I to stage III. Our study is in accordance with the study conducted by Malkin A and Co-investigators, they studied serum GGT along with carcino embryonic antigen, pregnancy associated macroglobulin and placental alkaline phosphatase in patients with ovarian and cervical cancer and concluded that, the frequency of GGT positivity increased with more advanced disease in cervical cancer and GGT reflected tumor burden in carcinoma of the cervix.[14]

Similar results were also documented by de Camargo and co-investigators in their study, the data showed GGT positive cells in cervical smears of patients with inflammatory or paraneoplastic or neoplastic conditions of the cervix. They concluded that cytochemically detectable transpeptidase activity appears whenever alterations of the normal epithelial microenvironment occurs.[86]

CONCLUSION

In conclusion, present study suggests that serum adenosine deaminase and gamma glutamyl transferase may be used as supportive parameters for diagnostic purpose and may add further for prognostic information. These biochemical parameters are inexpensive, rapid and can be easily analysed in smaller laboratories which have not exposed to any sophisticated technology.

However study on lipid peroxidation is required for correlating with release of membrane associated enzyme ADA into the circulation. Further studies on a larger sample with longer follow up are needed to substantiate our findings before firm conclusions can be drawn on the utility of these enzymes for the diagnosis and assessment of progression of cervical cancer.

SUMMARY

The results of the present study indicate that there was a significant increase in serum adenosine deaminase level as compared to controls. This could be due to the increased activity of ADA in cervical cancer as a result of local hypoxia which inhibits the cell mediated anti tumor immune response, promotes tumor cell migration and angiogenesis and stimulates the proliferation of tumor cells. As a result serum ADA activity is also increased to detoxify the high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in the cancerous tissues.

Serum gamma glutamyl transferase level was significantly increased in cervical cancer patients as compared to controls. The increased activity of serum GGT a microsomal glycoprotein enzyme could be due to alterations of the normal epithelial micro environment as a result of neoplatic transformation of the cervical epithelium.

BIBLIOGRAPHY

1. Department of Reproduction Health and Research. Progress in reproductive health Research, Vol No. 65. Geneva: World Health Organisation; 2004.

2. Shanta V, Krishnamurthi S, Gajalaxmi CK, Swaminathan R, Ravichandran K. Epidemiology of cancer of the cervix: Global and national perspective. J Indian Med Assoc 2000; 98(2): 49-52.

3. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. Cancer incidence in five continents. Vol . VII, Lyon France: IARC Scientific Publication No. 143; 1997.

4. Ferlay J, Bray F, Pisani P, Parkin DM, GLOBOCAN 2000: Cancer incidence, mortality and prevalence worldwide, version 1.0 IARC Press; 2001 available from:URL: http://www-dep.iarc.fr/globocan/globocan.htm Last updated on 03/02/2001Last Accessed: March 11th 2003

5. DiSAIA PJ, Creasman WT. Textbook of clinical gyanegologic oncology. 4th Ed. Baltimore: St. Louis; 1993.

6. Gajalaxmi CK, Krishnamurthy S, Anant R, Shanta V. Cervical cancer screening in Tamilnadu, India: A feasibility study of training the village health nurse. Cancer Causes Control. 1996; 7(5): 520-4.

7. Shermen ME, Schiffman M, Herrero R, Kelly D, Bratti C, Mango LJ, Alfaro M et al. Performance of a semiautomated papanicolaou smear

screening system: Results of a population based study conducted in Guanacaste, Costa Rica. Cancer 1988; 84: 273-80.

8. Majhi AK, Karmakar KS, Bhattacharya D. Chemoprevention of carcinoma cervix. Obs & Gynae Today 2005; 10(3): 166-70.

9. Basu P, Biswas J, Mandal R, Choudhury P. Is Interferon – α and retinoic acid combination along with radiation superior to chemoradiation in the treatment of advanced carcinoma of cervix. Indian Journal of Cancer 2006; 43(2): 54-9.

10. Wilkinson JH. The Principles and practice of diagnostic enzymology. London: Edward Arnold Publishers Ltd.; 1976.

11. Burtis CA, Ashwood ER. Tietz fundamentals of clinical chemistry. 6th Ed. New Delhi: Elsevier (India) Private Ltd.; 2008

12. Kato H, Niyoughi F, Morioka H, Fujino T, Torigoe T. Tumor antigen of human cervical squamous cell carcinoma: Correlation of circulating levels with disease progress. Cancer 1979; 43: 585.

13. van Nagell JR, Donaldson ES, Gay EC, Hudson S, Sharkey RM, Primus FJ et al. Carcinoembryonic antigen in carcinoma of the uterine cervix 2. Tissue localization and correlation with plasma antigen concentration. Cancer 1979; 44: 944.

14. Malkin A, Kellen JA, Lickrish GM, Bush RS. Carcinoembryonic antigen (CEA) and other tumor markers in ovarian and cervical cancer. Cancer 1978; 42: 1452-6.

15. Cortran RS, Kumar V, Collins T. Pathologic basis of disease. 6th Ed., Philadelphia: W. B. Saunders: 2003.

16. Stallworthy J, Bourne G. Recent advances in obstetric and gynaecology. 11th Ed. London: Churchill Livingstone; 1966.

17. Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, Heintz AP et al. Carcinoma of the cervix uteri. J Epidemiol Biostat. 2001; 6(1): 7-43.

18. Janicek MF, Averette HE. Cervical cancer: prevention, diagnosis, and therapeutics. CA Cancer J Clin. 2001; 51(2): 92-114.

19. Smith RA, Mettlin CJ, Davis KJ, Eyre H. American Cancer Society guidelines for the early detection of cancer. CA Cancer J Clin. Jan- 2000; 50(1): 34-49.

20. Morrow CP. Synopsis of Gynecologic Oncology. 5th Ed. NewYork: Churchill Livingstone; 1998.

21. DiSaia PJ, Creasman WT. Clinical Gynecologic Oncology. 5th Ed. St Louis: Mosby-Year Book; 1997.

22. Hoskins WJ, Perez CA, Young RC. Principles and Practice of Gynecologic Oncology. 2nd Ed., Philadelphia: Lippincott Raven; 1997.

23. Damjanov I, Linder J. Anderson's Pathology. 10th Ed. St Louis: Mosby-Year Book; 1996.

24. American Cancer Society. Cervical cancer: what are the key statistics about cervical cancer? Revised August 4, 2006. Available from: URL; http://www.cancer.org/docroot/CRI/content/CRI_2_4_1X_What_are_the_key_statistics_for_cervical_cancer_8.asp. Accessed August 14, 2008.

25. Ogunmodede F, Yale SH, Krawisz B, Tyler GC, Evans AC. Human papillomavirus infections in primary care. Clin Med Res. Dec 17 2007; epub ahead of print.

26. Cervical cancer: Revised October 13, 2008. Available from: URL; http://en.wikipedia.org/wiki/Cervical_cancer

27. Rock JA, Faro S, Gant NF, Horowitz JR, Moshy MAA. Advances in obstetrics and gynaecology. Chicago: Mosby Year Book; 1994.

28. Bieber EJ, Sanfili JS, Horowitz JR. Textbook of clinical gynaecology. Canada: Churchill Livingstone; 2006

29. Cancer: Fact Sheet No. 297. Geneva: World Health Organization; 2006.

30. Coppleson M, Monagham JM, Morrow GP, Tattersall MHN. Textbook of gynaecologic oncology. 2nd Ed. London: Churchill Livingstone: 1992.

31. Parkin DM, Loara E, Muir CS. Estimates of the worldwide frequency of sixteen major cancers. Int J Cancer 1988; 41: 184.

32. HPV Information centre. Human papilloma virus and cervical cancer: Summary Report. India: World Health Organization; 2007.

33. Shaw RW, Soutter WP, Stanton SL. Textbook of gynaecology. 2nd Ed. Tokyo: Churchill Livingstone; 1997.

34. Zur Hausen H. Papillomaviruses causing cancer: Evasion from host cell control in early event in carcinogenesis. J Natl Cancer Inst 2000; 92: 690-8.

35. Palefsky JM, Holly EA. Molecular virology and epidemiology of human papilloma virus and cervical cancer. Cancer Epid Biomarkers Prev 1995; 4: 415-28.

36. Havre PA, Yuan J, Hendrick L. P53 inactivation by HPV E16 results in increased mutagenesis in human cells. Cancer Res 1995; 55: 4420-4.

37. Tommasino M, Adamczewski JP, Carlotti F. HPV 16 E7 associates with the protein kinase p33CDK2 and cyclin A. Oncogene 1993; 8: 195-202.

38. Yokota J, Tsukada Y, Nakajima T, Gotoh M, Shimosato Y, Mori N et al. Loss of heterozygosity on the short arm of the uterine cervix. Cancer Res 1989; 49: 3598-601.

39. Riou G, Barrois M, Sheng ZM, Duvillard P, Lhomme C. Somatic deletions and mutations of c-Ha-ras gene in human cervical cancers. Oncogene 1988; 3: 329-33.

40. Sagae S, Kudo R, Kuzumaki N, Hisada T, Mugikura Y, Nihei T et al. Ras oncogene expressions and progression in intraepithelial neoplasia of the uterine cervix. Cancer 1990; 66: 295-301.

41. Agnantis NJ, Spnadidos DA, Mehere H, Parissi P, Kakkanas A, Pintzas A et al. Immunohistochemical study of ras oncogene expression in endometrial and cervical human lesions. Eur J Gynaecol Oncol 1988; 9: 360-5.

42. Sowani A, Ong G, Dische S, Quinn C, White J, Soutter P et al. C-myc oncogene expressions and clinical outcome in carcinoma of the cervix. Mol Cell Probes 1989; 3: 117-23.

43. Di Luca D, Costa S, Monini P, Rotola A, Terzano P, Savioli A et al. Search for human papillomavirus, herpes simplex virus and c-myc oncogene in human genital tumors. Int J Cancer 1989; 43: 570-7.

44. Baker V, Hatch K, Shingleton H. Amplification of the c-myc proto-oncogene in cervical carcinoma. J Surg Oncol 1988; 39: 225-8.

45. Ocadiz R, Sauceda R, Cruz M, Graef AM, Gariglio P et al. High correlation between molecular alterations of the c-myc oncogene and carcinomas of the uterine cervix. Cancer Res 1987; 47: 4173-7.

46. Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Padiatr 1998; 132: 277-84.

47. Nobbenhius MAE, Walboomers JMM, Helmerhost TJM, Rozendaal L, Remmink AJ, Risse EKJ et al. Relations of human papillomavirus status

to cervical lesions and consequences for cervical cancer screening: A prospective study. Lancet 1999; 354: 20 – 5.

48. DeVita VT, Hellman S, Rosenberg SA. Cancer: Principles and practice of oncology. 6th Ed. Philadelphia: Lippincott Williams and Wilkins; 2001.

49. Stoler MH, Schiffman M. Atypical Squamous cells of undetermined significance – low – grade squamous intraepithelial lesion Triage studyu (ALTS) group: Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS – LSIL Triage study. JAMA 2001; 285: 1500-5.

50. Kivlahan C, Ingram E. Papanicolaou smears without endocervical cells are they inadequate ? Acta Cytol 1986; 30: 258.

51. Norris HJ, Hertig AT, Abell MR. The uterus. International academy of pathology monogram. Baltimore: Williams and Wilkins; 1973.

52. Padubidri VH. Howkins and Bourse Shaws textbook of gynaecology. 13th Ed. New Delhi: Elsevier India Private Ltd.; 2006.

53. Flint A, McCoy JP, Schade WJ, Hofheinz DA, Haines HG. Cervical carcinoma antigen: Distribution of neoplastic lesions of the uterine cervix and comparison to other tumor markers Gynecol Oncol 1988; 30: 63.

54. Hussa RO, Mario T, Strobel JL, Pattilo RA, Mochizuki M. Production of tumor associated antigen, TA-4, by the Caski 1986;

55. Senekjian EK, Young JM, Weiser PA, Spencer CE, Magic SE, Herbst A. An evaluation of squamous cell carcinoma antigen in patients with cervical squamous cell carcinoma. American J Obstet Gynecol 1987; 157: 433.

56. Crombach G, Scharl A, Vierbuchen M, Wurz H, Bolte A. Detection of squamous cell carcinoma antigen in normal squamous epithelia and in squamous cell carcinomas of the uterine cervix. Cancer 1989; 63: 1337.

57. Duk JM, Aalders JG, Fleuren GJ, Krans M, De Bruijn HWA. Tumor markers CA 125, squamous cell carcinoma antigen and carcinoembryonic antigen in patients with adenocarcinoma of the uterine cervix. Obstet Gynecol 1989; 73: 661.

58. Dodd JK, Henry RJW, Tyler JPR, Houghton CRS. Cervical carcinoma: A Comparison of four potential biochemical tumor markers. Gynecol Oncol 1989; 32: 248.

59. Fujii S, Konishi I, Nanbu Y, Nonogaki H, Kobayashi F, Sagawa N et al Analysis of the elvels of CA 125 carcinoembryonic antigen and CA19-9 in the cervical mucus for detection of cervical adenocarcinoma. Cancer 1988; 62: 541.

60. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th Ed., Missouri: W. B. Saunders; 2006.

61. Coleman JM, Huffon JN. ADA activity in peripheral blood cells of patients with hematological malignancies. Br J Cancer 1976; 33: 312-9.

62. Bansal SK, Singh RP, Narang RK, Joshi LD, Bansal AB, Agarwal AK. Serum ADA activity in pulmonary tuberculosis, malignancy and non tubercular respiratory diseases. Indian J Chest Dis Allied Sci 1991; 33 (4): 189-93.

63. Franco R, Casada V. Cell surface adenosine deaminase much more than an ectoenzyme. Prog Nurobiol 1997; 52(4): 283-94.

64. Pratibha K, Anand U, Agarwal R. Serum adenosine deaminase 5' nucleotidase and malondialdehyde in acute infective hepatitis. Ind. J. Clin. Biochem 2004; 19(2): 128-31.

65. Ungerer JPJ, Oosthizen HM, Bissbort SH, Varmack WJH. Serum adenosine deaminase: Isoenzymes and diagnostic application. Clin Chem 1992; 38(2): 1322-6.

66. Ungerer JPJ, Oosthizen HM, Retief JH. Significance of ADA activity and its isoenzymes in tubercular effusions. Chest 1994; 106: 33-7.

67. Arokayam S. ADA: An aid to diagnose tuberculosis. J Med Sci 2003; 3(1): 30-45.

68. Franco R, Valenzuela A, Lenis C, Blanco J. Enzymatic and extra enzymatic role of ecto adenosine deaminase in lymphocytes. Immunol Rev 1998; 161: 27-42.

69. Murry RK, Granner DK, Mayes PA, Rodwll VW. Harpers biochemistry. 25th Ed. London: McGraw Hill; 2000.

70. Conwey EJ, Cooke R. The deaminases and adenine and adenylic acid in blood and tissues. Biochem 1939; 33: 479-92.

71. Vanderweyden MB, Kelly WN. Human ADA distribution and properties. J Clin Chem 1976; 251(18): 5448-56.

72. Gajdos A. Familial immune deficit and genetic deficiency of ADA of nucleotide phosphorylase 1. Nous Press Med 1978; 7: 929.

73. Sullivan J, Obsorue WRA, Wedgwood RJ. ADA activity in lymphocytes. Br J Hematol 1977; 37: 157-8.

74. Hovi T, Smyth JF, Allison AC, Williams SC. Role of ADA in lymphocyte proliferation. Clin Exp Immunol 1976; 1276: 395-403.

75. Satyanarayana U. Biochemistry. Calcutta: Calcutta Books and Allied (P) Ltd.; 2001.

76. Nyce JW. Insight into adenosine receptor function using antisence and gene knowckout approaches. Trends Pharmacol Sci 1999; 20: 79-83.

77. Kuyuee N, Karakurt C, Bilaloglu E, Karacan C, Tezic T. Adenosine deaminase in childhood pulmonary tuberculosis: Diagnostic value in serum. J Trop Ped 1999; 45: 245-7.

78. Borzenko BG. Activity of adenosine and thymidine metabolism enzymes in the blood of cancer patients of various ages. Ukr Biokhim Zh 1990; 62(1): 39-43.

79. Specchia G, Pavone V, Maggio F, Lojudice L, Iacobazzi A, De Tullio L et al. Adenosine deaminase activity in peripheral lymphocytes of patients with gynaecologic malignancies. Boll Ist Sieroter Milan. 1985; 64(5): 404-7.

80. Naidu SK, Suryankar AN, Swami SC, Katkam RV, Kumbar KM. Oxidative stress and antioxidant status in cervical cancer patients. Ind J Clin Biochem 2007; 22(2): 140-4.

81. Ishikawa M. Oxygen radicals – superoxide dismutase system and reproduction medicine. Nippon Sanka Fujinka Zasshi 1993; 45(8): 842-8.

82. Kim YT, Kim JW, Choi JS, Kim SH, Choi EK, Cho NH. Relation between deranged antioxidant system and cervical neoplasia. Int J Gynecol Cancer 2004; 14(5): 889-95.

83. Balasubramaniyan N, Subramanian S, Govindswamy S. Status of antioxidant systems in human carcinoma of uterine cervix. Cancer Lett. 1994; 87(2): 187-92.

84. Manoharan S, Kolanjiappan K, Kayalzizhi M. Enhanced lipid peroxidation and impaired enzymic antioxidant activities in the erythrocytes of patients with cervical carcinoma. Cell Mol Biol Lett 2004; 9: 699-707.

85. Henry JB. Clinical diagnosis and management by laboratory methods. 20th Ed. Philadelphia: W. B. Saunder Company; 2001.

86. de Camargo JL, de Oliveira ML, Mantovani MS, Gaiotto ME, de Mattos MC, Traman P. Cytochemical evaluation of gamma-glutamyl transpeptidase activity in cervical smears. Acta Cytol 1988; 32 (2): 148-52.

87. Bergmeyer HV. Method of enzymatic analysis. 2nd Ed., New York: New York Academic Press Inc; 1974.

88. International Federation of Clinical Chemistry. IFCC methods for the measurement of catalytic concentrations of enzymes. J Clin Chem Clin Biochem 1986; 24: 497.

ANNEXURE I – CONSENT FORM

Mrs/Ms. …… you are invited to participate in our research study that is a study to know the enzyme markers in carcinoma cervix.

Participation in this study is completely voluntary. About 40 patients and equal number of healthy females as volunteers will be enrolled in this study at J. N. Medical College, Belgaum under the supervision of Dr. P. B. Desai, Professor and Head, Department of Biochemistry, J. N. Medical College, K.L.E. University, Belgaum and will be carried out by Dr. Chetana K., P. G. Department of Biochemistry, K.L.E. University, Belgaum for her M.D. Thesis to be submitted to KLE University, Belgaum.

PURPOSE OF THE STUDY

The incidence and number of mortality due to carcinoma cervix are increasing in India. Hence this work is undertaken to assess the reliability of enzyme markers which are inexpensive, rapid and easily analysed in smaller laboratories.

PROCEDURE

For both carcinoma cervix patients (Cases) and healthy female subjects (Controls), 5 ml of venous blood will be collected under aseptic precautionary measures using sterile disposable syringe.

RISKS

Since the blood is drawn under aseptic precautionary measures by trained persons there is no scope for any risks. Further only small volume of blood is collected which will be spontaneously replenished in body. However there may

be minor risks associated with having blood drawn that may include bruising, redness, discomfort or bleeding at the puncture site.

BENEFITS

No direct benefit is guaranteed to you from participating in our study. You can make use of blood levels of studied parameters if desired.

OPTIONS

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

NEW INFORMATION

Does not apply to this research.

PRIVACY AND CONFIDENTIALITY

All information collected about you during the course of the study will be kept confidential to the extent permitted by law. You will be identified in this research record by the code numbers. Information which identifies you personally will not be revealed without your written permission. However your records may be revealed to the sponsor of the study. Information from this study may be published but your identity will be confidential in any publication.

INSTITUTIONAL POLICY

In the event that you are physically injured as a result of participating in this research emergency care will be available. There is no commitment to provide any compensation for research related injury. The J. N. Medical College will provide, within the limitations the laws of the state if Karnataka, facilities and medical attention to subjects who suffered any harm as the result of your participation in this study. In the event you believe that you have suffered any

how as a result of your participation in this study you may contact research guide Dr. P. B. Desai, Professor and Head, Department of Biochemistry.

COST FOR PARTICIPATION

You will not be charged for the test to be carried out on your blood sample.

FINANCIAL INCENTIVE FOR PARTICIPATION

You will not receive any remuneration for participating in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Dr. V. D. Patil, Principal, J. N. M. C., K.L.E. University, Belgaum and Chairman of J. N. M. C. Institutional Ethics Committee of Human Subjects Research, Telephone No. 0831-2471701.

EMERGENCY PROVISION

If you have questions as a participant in our study, you can contact the study investigator Dr. Chetana K., Mobile No. 94489 22664 or the research guide Dr. P. B. Desai, Phone No. 0831-2473777 (Extension) 1522.

CONSENT TO PARTICIPATE IN A RESEARCH TRIAL

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving a any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

_____ _____

Signature of participant Date

_____

Participants Name (Printed):

_____ _____

Name and Signature of witness-1 Date

_____ _____

Name and Signature of witness-2 Date

_____ _____

Signature of researchers or Date
Person obtaining consent

ANNEXURE II – PROFORMA

"EVALUATION OF ENZYME MARKERS IN CERVICAL CANCER – A CROSS SECTIONAL STUDY"

I. Sl. No.: IP / OP No.: _____

Name: _____ DOA: _____

Age: _____ Occupation: _____

Religion: _____

Address _____

II. History of present Illness

WDPV: PV Bleeding:

Mass per vagina: Mass per Abd:

Pain Abdomen: Backache:

Infertility:

Menstrual History

Menarche: _____ Yrs. Menopause: _____ Yrs

		Days	Flow
LMP	Pa MC	-	
	Pr MC	-	

Obstetric history

Married Life: Yrs

If pregnant, period of gestation months

Last delivery: Yrs back

H/o multiple sexual partners:

III. Past History

H/o of TB, DM, HTN or any other major illness.

IV. Personal history

Diet:

Sleep:

Appetite:

Habits: Alcohol, Smoking, Tobacco chewing

Bowel and bladder

Frequency of micturation:

Dysuria:

Haematuria:

Diarrhoea:

Rectal pain:

Bleeding PR:

V. General physical examination

Vital signs: Pulse: RR:

Temp: BP:

Nourishment: Edema:

Height: Weight:

Nails:

Lymphadenopathy:

Pallor:

Icterus:

Cyanosis:

VI. Systemic examination

a. Obstetric examination

P/A:

P/S:

P/V:

b. Other systems

Respiratory system:

Cardiovascular system:

Central nervous system:

Others:

VII. Investigations

Serum ADA: U/L

Serum GGT: U/L

VIII. Histological diagnosis

IX. Final Diagnosis

ANNEXURE III – MASTER CHART

CONTROLS

Sl. No.	Name	Age (Years)	ADA (U/L)	GGT (U/L)
1	B.W.	54	25.00	19.68
2	K.B.	50	20.00	20.80
3	M.L.	56	20.00	18.52
4	F.K.	38	10.00	34.74
5	S.B.	60	26.00	12.70
6	L.S.	42	16.66	17.37
7	V.L.	48	16.66	22.00
8	P.S.	45	16.66	17.37
9	A.L.	65	25.00	12.70
10	R.K.	68	25.00	12.70
11	P.T.	53	20.00	18.5
12	S.T.	51	20.00	22.00
13	S.V.	47	20.00	17.37
14	S.N.	48	20.00	22.00
15	P.N.	40	25.00	17.30
16	M.D.	44	20.00	15.00
17	R.B.	55	25.00	13.80
18	S.G.	56	25.00	13.80
19	C.N.	35	10.00	34.70
20	P.F.	56	25.00	18.52

Sl. No.	Name	Age (Years)	ADA (U/L)	GGT (U/L)
21	G.M.	54	25.00	13.80
22	M.L.	44	16.60	18.50
23	Y.L.	45	20.00	17.37
24	M.W.	54	25.00	13.80
25	N.S.	46	16.60	22.00
26	A.N.	39	10.00	22.00
27	T.W.	38	10.00	13.89
28	M.G.	57	33.30	16.20
29	B.M.	60	30.00	17.30
30	S.N.	57	25.00	13.89
31	K.K.	40	25.00	25.47
32	S.S.	55	30.00	24.30
33	G.S.	37	10.00	25.47
34	M.W.	52	25.00	13.89
35	M.I.	45	25.00	18.50
36	M.D.	52	25.00	24.31
37	P.T.	55	25.00	25.47
38	S.C.	56	20.00	18.50
39	S.H.	38	10.00	35.80
40	S.K.	56	25.00	18.52

CASES

Sl. No.	Name	Age (Years)	Stage of Cervical Cancer	ADA (U/L)	GGT (U/L)
1	N.B.	35	Stage I	83.33	38.21
2	B.W.	48	Stage I	33.33	26.63
3	N.G.	56	Stage I	33.33	30.10
4	S.M.	40	Stage I	66.66	35.89
5	B.W.	55	Stage I	33.33	38.20
6	P.T.	40	Stage II	50.00	34.74
7	K.M.	60	Stage II	50.00	23.16
8	Y.M.	40	Stage II	50.00	37.05
9	A.S.	53	Stage II	50.00	28.95
10	H. M.	60	Stage II	50.00	46.32
11	A.M.	54	Stage II	50.00	42.80
12	S.B.	35	Stage II	50.00	63.69
13	K.H.	55	Stage II	50.00	41.60
14	S.P.	38	Stage II	33.30	38.20
15	A.M.	55	Stage III	66.66	35.89
16	G.W.	50	Stage III	50.00	42.80
17	S.H.	38	Stage III	33.35	41.60
18	S.K.	50	Stage III	50.00	38.20
19	P.M.	60	Stage III	66.60	44.00
20	I.M.	50	Stage III	66.60	39.30

Sl. No.	Name	Age (Years)	Stage of Cervical Cancer	ADA (U/L)	GGT (U/L)
21	S.K.	50	Stage III	83.33	38.20
22	Y.H.	40	Stage III	66.66	36.60
23	S.L.	40	Stage III	50.00	36.60
24	A.N.	45	Stage III	33.33	40.53
25	H.G.	35	Stage III	33.33	42.80
26	N.M.	65	Stage III	50.00	39.30
27	P.A.	47	Stage III	66.66	38.20
28	S.M.	42	Stage III	66.66	41.00
29	N.L.	55	Stage III	50.00	62.53
30	S.M.	38	Stage III	50.00	37.05
31	B.S.	38	Stage III	50.00	56.74
32	I.L.	45	Stage III	50.00	44.00
33	P.T.	50	Stage III	75.00	50.95
34	S.K.	55	Stage III	100.00	39.37
35	K.N.	50	Stage III	50.00	41.68
36	G.M.	45	Stage III	125.00	50.95
37	J.R.	60	Stage III	75.00	57.90
38	L.M.	50	Stage III	50.00	39.30
39	S.T.	68	Stage III	50.00	39.30
40	K.V.	48	Stage III	66.66	38.20

KEY TO MASTER CHART

ADA	-	Adenosine deaminase
GGT	-	Gamma glutamyl transpeptidase
Sl. No	-	Serial number
U/L	-	Units per liter